

50

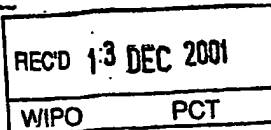
Document # 5

Opposition against EP 1 220 893
Application No. 00 969 319.3
Proprietor: Baxter Aktiengesellschaft
Opponent: Merck Serono International S.A.



Europäisches
Patentamt

European
Patent Office



Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

00120896.6

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

DEN HAAG, DEN
THE HAGUE,
LA HAYE, LE

03/12/01



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: **00120896.6**

Anmeldetag:
Date of filing:
Date de dépôt: **25/09/00**

Anmelder:
Applicant(s):
Demandeur(s):
Polymun Scientific Immunbiologische Forschung GmbH
1190 Wien
AUSTRIA

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Influenza vaccine and method of manufacture

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:
State:
Pays:

Tag:
Date:
Date:

Aktenzeichen:
File no.
Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification Internationale des brevets:
C12N7/00, A61K39/145

Am Anmeldetag benannte Vertragsstaaten:
Contracting states designated at date of filing: **AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LU/MC/NL/PT/SE/TR**
Etais contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

BEP-4847-EU

- 1 -

INFLUENZA VACCINE AND METHOD OF MANUFACTURE

TECHNICAL FIELD

5 The present invention is in the field of virology and vaccine development and relates to novel Influenza vaccines based on attenuated, particularly cold adapted and temperature sensitive, influenza A and B strains.

10 The invention further relates to a simple and efficient process for isolating viruses from various sources and for producing live attenuated Influenza vaccines in a serum-free Vero cell culture under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or prevented, and to vaccines derived therefrom.

15 BACKGROUND OF THE INVENTION

The influenza hemagglutinin (HA) antigen is the major target for the protective immune responses of a host to the virus.

20 A common practice of recovering new viral isolates involves recovery from a nasal or throat swab or from a similar source, followed by cultivation of the isolates in embryonated chicken eggs. The virus adapts to its egg host and large scale production of the virus can be carried out in eggs. Such conventional methodology involving embryonated chicken eggs to produce Influenza vaccine is, however, extremely cumbersome, involving the handling of many thousands
25 of eggs per week as well as extensive purification of the virus suspension derived from the allantoic fluid to ensure freedom from egg protein.

Another disadvantage in the use of chicken embryos for virus production lies in the fact that this substrate strongly favors the selection of virus variants that
30 differ in their antigenic specificity from the wildtype virus and not rarely results in viruses that may not be suitable for vaccine production due to their altered phenotypes including, for instance, considerable reduction in immunogenicity.

Many attempts have therefore been undertaken in the art to utilize standard
35 tissue culture technology with established mammalian cell lines, such as MDCK (Madin-Darby Canine Kidney) or Vero (African Green Monkey Kidney) cells, for virus production, particularly influenza virus production.

- 2 -

One of the difficulties in growing influenza strains in tissue cell culture arises from the necessity for proteolytic cleavage of the influenza hemagglutinin in the host cell. Cleavage of the virus HA precursor into the HA1 and HA2 subfragments, although not necessary for the assembly of the viral elements to form a complete virion, is required, however, to render the virion infective, i.e. to enable it to infect a new cell.

It has been reported (e.g. Lazarowitz et al., "Enhancement of the Infectivity of Influenza and B Viruses by Proteolytic Cleavage of the Hemagglutinin Polypeptide", Virology, 68:440-454, 1975) that the limited replication of several Influenza A strains in standard cell cultures could be overcome by the addition of proteases like trypsin to the tissue culture medium. Yet, there remained difficulties in some cases, for instance when using Vero cells.

Kaverian and Webster (J Virol 69/4:2700-2703, 1995) report that in Vero cell cultures, and less pronounced in MDCK, swine kidney, or rhesus monkey kidney cell cultures, the trypsin activity in the medium rapidly decreased from the onset of incubation resulting in the failure of virus accumulation in the medium due to the lack of production of a sufficient number of infective virions. They concluded that a trypsin inhibiting factor was released from the Vero cells. They further showed that by repeated addition of trypsin reproduction of virus could be resumed and maintained for a number of reproduction cycles resulting in a much better virus yield.

Another way for efficient vaccine production was reported in US 5,753,489 wherein serum-free medium was used for virus propagation in a number of different mammalian cells including MDCK and Vero cells. The method disclosed therein comprises growing vertebrate cells in serum-free medium, infecting the cell culture with a virus, incubating the cell culture infected with the virus, removing a portion of the virus-containing medium and contacting this portion with a protease, thereafter adding to that portion a protease inhibitor and returning that portion to the cell culture. It is preferred therein to provide the steps of growing, infecting and incubating in a first vessel and the steps of trypsin-contacting and inhibitor-adding are performed in a second vessel connected with the first vessel in a loop so that the steps can be performed in

- 3 -

a closed cycle. This system allows to use trypsin or other proteolytic enzymes at much higher concentrations than those normally tolerated by cells in culture.

Experiments leading to the present invention have shown, however, that even
5 between mammalian cells such as MDCK and Vero cells, there exist differences with regard to antigenic variations of the virions produced on such host cell systems.

SUMMARY OF THE INVENTION

10

It is therefore an object of the present invention to provide for a method for the production of viruses, particularly Influenza viruses, that yields viral progeny that selectively agglutinates human erythrocytes but not chicken erythrocytes, and that preferably has antigenic properties identical with those of the initially
15 inoculated virus strain, e.g. the primary clinical wildtype isolate.

In a preferred embodiment, the nucleic acid sequence of the HA gene and optionally of the NA gene of the propagated virus is identical with the one of the initially inoculated strain (e.g. an epidemic strain, primary clinical isolate of
20 an infected patient).

It is another object of the invention to provide a method for efficient production of a live attenuated vaccine in a single step procedure that does not require any purification steps of the virus suspension harvested from the cell culture
25 supernatant by centrifugation.

It is yet another object of the invention to provide attenuated, cold adapted and temperature sensitive Influenza A and B strains and vaccines made thereof.

30 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a Vero cell culture.

35 Fig. 2 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a MDCK cell culture.

- 4 -

DETAILED DESCRIPTION OF THE INVENTION

As mentioned above, the host system is decisive for the antigenic properties of viruses propagated therein. Comparative experiments using embronated eggs,

- 5 MDCK and Vero cells clearly proved that the initially inoculated virus is likely to undergoe antigenic alteration during growth on any one of these substrates

Evidence has accumulated, however, that the alterations are least or even absent for influenza virus strains grown on Vero cells in serum-free medium.

- 10 Moreover, it turned out that influenza A viruses, at least strains of the H3N2 subtype, exhibit a selectivity for agglutination of human erythrocytes but not for chicken erythrocytes. Also, they did not grown on eggs. This was a first indication that these Vero-grown viruses might be more identical with the wildtype virus of the corresponding clinical isolate than the ones grown on
- 15 MDCK cells or eggs.

Indeed, comparison of the HA and NA gene sequences of wildtype isolates obtained from nasal swabs with the ones of the same viruses after growth on Vero and MDCK cells, respectively, revealed alterations in the HA or NA of

20 MDCK-grown viruses relative to the swab isolates or Vero-grown viruses or both.

- Moreover, experimental data obtained from Immunizations of ferrets with Vero- and MDCK-grown wildtype viruses indicate a far stronger virulence of the Vero-
- 25 grown viruses compared to the MDCK-grown viruses. Also, the immunogenicity of the Vero-grown viruses tested in an animal trial on macaques was demonstrated to be significantly superior to the one of the MDCK- or egg-grown viruses.

- 30 These findings together provide strong evidence for the hypothesis that the process for the multiplication and propagation of viruses according to the present invention as hereinafter described in more detail yields viruses that are either unaltered compared to the initially inoculated (e.g. wildtype) virus or are modified to only a minor extent that was not known or achieved in the art
- 35 hitherto.

- 5 -

It is not only the avoidance of antigenic alterations that makes the present process of virus multiplication so unique, but it is also its striking simplicity which makes it extremely suitable for large scale industrial vaccine production. It is therefore an object of the present invention to provide a process for the manufacture of an influenza vaccine that is simple, highly efficient and yields a safe, attenuated live vaccine.

- Further experiments have shown that the source of trypsin (or trypsinogen) may be one additional factor that influences the overall yield of infective virions.
- 10 Indeed, while the methods known in the art (e.g. Kaverin and Webster, J Virol 69/4:2700-2703, 1995; or US 5,753,489) use either repeated addition of trypsin (Kaverin and Webster) or high trypsin concentrations (US 5,753,489), the process according to the present invention applies only half or less of the trypsin concentrations reported in the prior art. Moreover, a single addition of as
- 15 little as 0.5 - 10 μ g, preferably 2 - 5 μ g trypsin per ml to the cell culture medium is sufficient to reach optimal infective virus titers. Inactivation experiments revealed that porcine or human recombinant trypsins are far less susceptible to inactivation by Vero or MDCK cells than bovine trypsin. Since bovine trypsin is most commonly used in the art it is rather likely that prior art
- 20 literature unless explicitly mentioning another trypsin source, implicitly refers to bovine trypsin only. This would also help to explain the modes and concentrations of trypsin application recited, for instance, in Kaverin et al. and in US 5,753,489.
- 25 Using porcine or human rec trypsin for supplementing the serum-free medium for Vero cell cultures according to the present invention therefore allows to use extremely low trypsin concentrations and thus prevent the need of labor-intensive and costly purification steps after harvesting of the virus-containing supernatant.
- 30 Another step that contributes to make the present process simple and therefore attractive to vaccine manufacturers is the addition of a single dose of highly active endonuclease to the cell culture medium at the beginning of incubation of the infected Vero cells for virus propagation. This endonuclease, preferably
- 35 BenzonaseTM, is added once to the medium at a very low concentration of 2 - 30, preferably 5 - 15, Units per ml of medium and effectively clears the cell culture medium from free DNA and RNA originating mainly from the lysing or

- 6 -

lysed host cells. The residual Benzonase enzyme concentration in the ready-for-use vaccine preparations obtained from the centrifuged supernatant remains at 5 ng or less per dose.

- 5 BenzonaseTM is a trademark of Nycomed Pharma A/S Denmark and relates to an extracellular unspecific endonuclease obtained from *Serratia marcescens*. Benzonase is a genetically engineered endonuclease which degrades both DNA and RNA strands in many forms to small oligonucleotides. It promotes quick reduction of the viscosity of cell lysates, which facilitates ultracentrifugation. It
- 10 reduces proteolysis and increases the yield in targeted protein and offers complete elimination of nucleic acids from, e.g. recombinant, proteins. It has an exceptionally high activity of 400,000 U/mg.

- A third and important advantage of the present process is the factor time hence
- 15 process costs. Due to the use of serum-free medium that does not contain proteins of animal origin nor antibiotics, expensive and time-consuming purification procedures can be reduced to a minimum or even totally avoided. Also, as the addition of exogenous enzymes such as the protease (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) occurs once at the beginning of
- 20 the virus propagation phase this saves plenty of time that the state-of-the-art methods require for post-incubation treatment of the virus-containing culture supernatant (e.g., HA activation, RNA/DNA digestion, protein purification, etc.). Surprisingly, it turned out that the early addition of either or both of trypsin and Benzonase to the virus-infected Vero-cell culture had no negative implications
- 25 on the virus yield, which is probably due to the very low enzyme concentrations applicable in the process of the present invention.

- The present process of virus propagation is particularly useful for the multiplication of influenza A viruses of the H3N2 subtype, but is of course also
- 30 suitable for the isolation and reproduction of any epidemic or laboratory influenza virus strain, regardless of the kind of virus inoculum (e.g., blood serum sample, nasal wash, nasal swab, pharyngeal swab, saliva, etc.). Using the principles of this process, a number of Influenza A and B vaccines has been produced which are part of the present invention and which are characterized
- 35 in more detail in the subsequent Examples. Also, protective efficacy as well as vaccine safety have been confirmed for the vaccines made according to the present invention, as will be demonstrated in the Examples.

- 7 -

In order that the invention described herein may be more fully understood, the following Examples are set forth. They are for illustrative purposes only and are not to be construed as limiting this invention in any respect.

5 Example 1: Virus production

Cultivation of Vero /SF (=serum-free) cells:

10 SF-Medium: DMEM (Biochrom F0435), Ham's F12 (Biochrom F0815), 5mM L-Gln, 0.1% SF-supplement (a) or (b); antibiotics (only for first passage of virus isolation).

SF-Supplement: protein hydrolysate of non-animal origin, without functional proteins such as Insulin, transferrin or growth factors:

15 a) 62.5 g hy-soy/UF, Quest 5X50100, to 500 g HQ-water, filtered with PES 0.2 µm filter;
b) 12.5 g hy-pep 1510, Quest, to 100 g HQ-water, filtered with PES 0.2 µm filter.

20 The content of a deep frozen (liquid nitrogen) disinfected (70% ethanol) ampule of WCB Vero cells was thawed and added to 9 ml of cold serum-free (SF) medium in a 10 ml tube and centrifuged for 10 min at 1000rpm (170 g). The pellet was resuspended in SF-medium to a total of 30 ml, transferred to a 80 cm² Roux bottle and incubated at 37°C and 7%CO₂ for at least 15 min.

Thereafter, the medium was removed and the cells were washed with approx. 25 0.1 ml/cm² PBS def. (= PBS without Ca²⁺ and Mg²⁺). Addition of trypsin/EDTA-solution (8-10 µl/cm²; 0.1% trypsin / 0.02% EDTA-solution) and incubation at room temperature for about 3 min. Detaching by gently pushing the Roux bottle against palm of the hand, addition of SF-medium and trypsin inhibitor (Sigma, T6522) at a quantity of about 1/5 of volume of the

30 trypsin/EDTA solution. Repartition of the cell suspension to Roux bottles or roller bottles, incubation at 37°C and 9% CO₂.

MDCK cells were grown in DMEM/Ham's F12 + 2% FCS (heat inactivated); embryonated hen eggs were 11-12 days old and of SPF (specific pathogen free) 35 origin.

- 8 -

Propagation of virus strains:

- Old medium from roller bottles containing Vero cells was removed and cells were infected with virus by addition of 5 ml virus suspension in SF-medium to each roller bottle, resulting in an MOI (multiplicity of infection) of approximately 0.01. After incubation for 45 minutes at 33°C the virus inoculum was removed with a pipette. 90ml of SF-medium supplemented with 0.5 - 10, preferably 2 - 5 and most preferably 2 µg/ml porcine trypsin (supplier: AvP) or human recombinant trypsin or trypsinogen (own production) and 0.5 g/l sodium bicarbonate were added to each roller bottle and the bottles incubated at 33°C and 5% CO₂. For the production of attenuated live vaccine samples for use in animal testing and in human clinical trials the SF-medium was supplemented with trypsin and, additionally, with BenzonaseTM at a concentration of 2 - 30, preferably 5 - 15, and most preferably 10 Units of BenzonaseTM per ml of medium. Virus was harvested after 64 hours post infection by centrifugation of the culture supernatant for 5 min at 4000 rpm (3000g) at 10°C in 50 ml-tubes. The supernatant was pooled for each virus strain and stored at +4°C. Aliquots thereof were used for vaccine testing.
- For storage purposes the virus preparations may be freeze-dried and stabilizer such as, for example, trehalose and lactalbumin enzymatic hydrolysate in HEPES buffer may be added. Reconstitution may be done with sterile water.

Example 2: Comparison of trypsin inactivation in cell cultures

25

Table 1: Trypsin inactivation

	0 h	24 h	48 h	72 h
bovine trypsin	0.314	0.189	0.11	0.026
porcine trypsin	0.23	0.201	0.171	0.133
porcine trypsin	0.129	0.108	0.081	0.054
human rec trypsin	0.097	0.054	0.026	0.008

- Supernatants obtained from uninfected Vero cell cultures (grown in SF medium as described in Example 1) and MDCK cell cultures (grown in FCS-supplemented medium as described in Example 1) were tested for their capacity to inactivate trypsin of different origin that has been added to the supernatant at time = 0 h at equal concentrations each. Porcine trypsin has been applied in two different

- 9 -

qualities (obtained from different manufacturers). The results are presented in Table 1 and in Figures 1 and 2.

The data unambiguously show that bovine trypsin is rapidly inactivated in Vero cell culture supernatant and less rapidly in MDCK cell culture supernatant. Porcine and human rec trypsin (manufactured in our laboratories) remain fully active in MDCK supernatants while they are gradually inactivated in Vero supernatants at approximately half or less of the velocity of bovine trypsin inactivation. The difference of the porcine trypsins tested is only in the starting OD-level at 247 nm, while the inactivation characteristics are essentially identical for both lots of porcine trypsin.

Example 3: Comparison of various viral properties after growth on different host cell substrates

Virus propagation was carried out as described in Example 1 for the different host cell substrates.

Table 2: Characteristics of H3N2 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs
			chicken erys	human erys	
A/47/96	A/Johannesburg/33/94	Vero	-	+	-
		MDCK	+	+	+
A/7729/98	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1143/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1144/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1179/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1180/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1182/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+

- 10 -

Each of the seven isolates recovered on Vero cells was reactive with human erythrocytes but not with chicken erythrocytes and none of them accumulated in embryonated eggs. On the other hand, all isolates recovered on MDCK cells were reactive both with chicken and human erythrocytes and were capable of growing in eggs. Although these differences were not seen in influenza A viruses of the H1N1 subtype nor in Influenza B isolates (see subsequent Tables 3 and 4), it may nevertheless be assumed that cultivation of influenza viruses on Vero cells will maintain antigenic properties more properly than cultivation on other substrates.

10

Table 3: Characteristics of H1N1 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		225
A/5389/95	A/Bayern/7/95	Vero	+	+	+	D
		MDCK	+	+	+	D
A/1035/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	G
		Swab	+	+	+	D
A/1131/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Swab	+	+	+	D
A/1134/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	n.t.
		Swab	+	+	+	D

From the data in Table 3 it appears that H1N1 influenza viruses may be less susceptible to adaptive selection, as the Vero and MDCK-grown isolates do not exhibit significant differences in their hemagglutination characteristics nor in their HA sequences. A similar conclusion may be drawn for the B isolates listed in Table 4.

- 11 -

Tabelle 4: Characteristics of B viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position 198
			chicken erys	human erys		
B/4291/97	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/1/99	B/Beijing/184/93	Vero	+	+	+	T(g.s)
		MDCK	+	+	+	T(g.s)
		EGG	+	+	+	A
		Swab				T(g.s)
B/110/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/147/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/156/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/157/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	

The clinical starting material (e.g. serum samples, swabs) for virus isolation and replication was primarily obtained from:

1. Institute of Virology, Vienna, Austria (Prof. F. Heinz) 1996/96, 1996/97
2. Unité de Génétique Moléculaire des Virus Respiratoires, Institute Pasteur, Paris, France (Prof. S. van der Werf) 1996/97
3. Public Health Laboratory Service, London, UK (Dr. M. Zambon) 1996/97
4. Laboratoire Central de Virologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland (Dr. W. Wunderli) 1996/97, 1997/98
5. Virus Unit, Queen Mary Hospital, Hong Kong (Dr. W.L. Lim) 1997/98

- 12 -

Table 5: Amino acid changes in HA, NA and M proteins of H3N2 influenza viruses isolated on different host systems

Isolate number	Changes at positions								
	HA						NA		M
	128	129	229	133	218	220	136	151	
A/47/96 Vero	T(g.s)								
A/47/96 MDCK	A								
A/7729/98 Vero		E	R						
A/7729/98 MDCK		G	K						
A/1143/99 Swab				N(g.s)	G		n.t	n.t	n.t
A/1143/99 Vero				N(g.s)	G			D	Identical
A/1143/99 MDCK				D	E			G	
A/1144/99 Swab						R	n.t		n.t
A/1144/99 Vero						R	Identical		Identical
A/1144/99 MDCK						G			
A/1179/99 Swab	Identical						n.t		n.t
A/1179/99 Vero							Identical		Identical
A/1179/99 MDCK									
A/1180/99 Swab	Identical						n.t	n.t	n.t
A/1180/99 Vero							Q		Identical
A/1180/99 MDCK							R		
A/1182/99 Swab	Identical						n.t		n.t
A/1182/99 Vero							n.t		n.t
A/1182/99 MDCK							n.t		n.t

The results show that with some isolates there was no alteration of the HA sequence of Vero or MDCK propagated viruses over the HA sequence directly obtained from the swab material by PCR amplification. In some other isolates grown on MDCK cells the HA and/or NA sequences were deviating from the corresponding sequences obtained on Vero cells. The Vero-derived viruses did not show, however, any deviations in the HA sequence over the HA sequence of the swab isolates, where determined.

- 13 -

Table 6: Immunogenicity of Vero-, MDCK- and Egg-derived viruses for macaques

Animal number	Virus for immunization	Dose, PFU/ml	Serum HI titers
96	A/Vienna/47/96 V	5×10^4	256
88	A/Vienna/47/96 V	5×10^4	128
15	A/Vienna/47/96 V	1.0×10^6	128
95	A/Vienna/47/96 V	1.0×10^6	256
93	A/Vienna/47/96 M	1.0×10^6	16
128	A/Johannesburg/33/94 E	5×10^8	32
110	A/Vienna/157/97 V	5×10^4	128
78	A/Wuhan/359/95 E	5×10^8	32

The Macaques were immunized i.n. in the absence of anesthesia with 1 ml of virus suspension

- 5 V - Vero- isolated virus
M - MDCK -isolated viruses
E - egg isolated viruses

Table 7: Virulence of Vero- and MDCK- derived variants of A/Vienna/47/96 wt virus for ferrets

10

Viruses	Virus dose, PFU/ml	Number of animals with fever on day		
		1	2	3
A/Vienna/47/96 Vero	2×10^2	FF	FFF	
	1×10^3	FFF	FFF	
A/Vienna/47/96 MDCK	5×10^2			
	5×10^3		FF	
	5×10^4	FF	F	F

Animals were immunized i.n. under ether narcosis with 1 ml of virus suspension.

N- normal temperature from 38.1°C to 39.9°C ;

F- fever, more than 40.0°C .

- 15 The most surprising, yet important result in Table 6 is the very low immunogenicity of MDCK-derived A/Vienna/47/96 virus compared with the corresponding Vero-derived virus. It is no particular surprise that the egg-derived viruses show only poor immunogenicity.

- 14 -

Similarly, the results listed in Table 7 indicate that Vero-derived viruses are less if at all altered by adaptive selection on their host substrate than the MDCK-derived viruses. This means that the Vero-derived viruses maintain more or even all of the immunologically relevant, particularly antigenic, properties of the
5 original virus than is the case with the MDCK-derived viruses.

Example 4: Vaccine production with preferred strains

The process described in Example 1 was also used for the production of vaccine
10 samples for animal testing and human clinical studies. It is understood that the process of virus propagation described therein also encompasses variations that could be suggested or applied by a person of ordinary skills in the art without inventive input and as long as the variations do not change the sense of the present invention as described herein and in the claims.

15
Vaccine samples containing one or more of the preferred influenza A or B wildtype strains, master strains or reassortant strains (that are subsequently described in more detail) were exclusively produced using the continuous Vero cell line as the host cell system (unless for purposes of comparison with
20 samples obtained from other host substrates) in serum-free medium additionally supplemented with the nutritional ingredients and enzymes as described in Example 1.

The methods used to modify the wildtype viruses to arrive at the preferred
25 influenza master strain candidates of the present invention including the methods of attenuation (e.g., temperature sensitivity), cold adaptation and reassortment are known in the art and extensively reviewed, for instance, in WO 99/64068.

30 Further characteristics of the two most preferred influenza A and B master strain candidates useful for attenuated live vaccine production, e.g., by 6/2 reassortment with the HA and NA genes of actual epidemic influenza viruses recommended by the WHO, are given in the following Tables B - 13.

- 15 -

Table 8: Characteristics of master strain candidates for live influenza vaccines

	Influenza A <i>A/Singapore/1/57/ca</i> H2N2	Influenza B <i>B/Vienna/1/99/ca</i>
Passage history	<i>A/Singapore/1/57</i> wt egg derived H2N2 20 passages at 37°C on Vero/SF cells 25 passages at 25°C on Vero/SF cells	<i>B/Vienna/1/99</i> wt Vero derived 1 additional passage at 33°C on Vero/SF cells 22 passages at 25°C on Vero/SF cells
Method of attenuation	Serial passages at optimal and suboptimal temperature on heterologous system	Serial passages at optimal and suboptimal temperature on heterologous system
Phenotypic markers	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs
Genotypic markers	Mutations: 13 (8 coding) PB2 3 (2 coding) PB1 2 (1 coding) PA 4 (3 coding) NP 1 M 2 (2 coding) NS 1	Mutations: 5 (3 coding) PB2 0 PB1 1 PA 0 NP 2 (1 coding) M 1 NS 1

Table 9: Full Sequence of the 8 genome segments and of the 10 corresponding proteins of strain *A/Singapore/1/57/ca*

<i>A/Singapore/1/57/ca</i> (H2N2)			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 1	PB2	SEQ ID No. 9
2	SEQ ID No. 2	PB1	SEQ ID No. 10
3	SEQ ID No. 3	PA	SEQ ID No. 11
4	SEQ ID No. 4	HA	SEQ ID No. 12
5	SEQ ID No. 5	NP	SEQ ID No. 13
6	SEQ ID No. 6	NA	SEQ ID No. 14
7	SEQ ID No. 7	M1	SEQ ID No. 15
		M2	SEQ ID No. 16
8	SEQ ID No. 8	NS1	SEQ ID No. 17
		NS2	SEQ ID No. 18

- 16 -

oa - cold adapted

It shall be noted, however, that the genome segments No. 4 and 6, i.e., the HA and NA genes, are not required to characterize the Influenza A master strain candidates, because these genes will be exchanged for the corresponding genes of actual epidemic influenza viruses (as mentioned hereinbefore). The features important for the safety of a vaccine, e.g. temperature sensitivity, or features that allow intranasal (where average temperature is lower than usual body temperature) administration of a vaccine, namely cold adaptation, are primarily caused by mutations in the remaining 6 genome segments.

The following Table 10 lists the mutations in the genome segments of A/Singapore/1/57/ca compared to the corresponding wildtype strain A/Singapore/1/57/wt

Table 10: Mutations in the genome segments of attenuated, temperature sensitive, cold adapted Influenza

strain A/Singapore/1/57/ca compared to A/Singapore/1/57/wt strain

RNA segment	Length (n'ds)	Nucleotides changed position	wt	ca	Protein	Length (aa)	Amino acids changed position	wt	ca
1	2341	252 581* 1048*	a t g	g c t	PB2	771	- 185 340	- I R	- T I
2	2341	1279* 1985	t a	a c	PB1	767	419 -	L -	I -
3	2233	707* 1425 1537* 1819*	a t a g	t a g c	PA	716	228 - 505 598	I - V Q	N - I E
5	1565	210	g	a	NP	508	-	-	-
7	1027	327* 499*	g g	a c	M1 M2	252 87	101 158 -	R Q -	K R -
8	890	813	a	g	NS1 NS2	237 121	- -	- -	- -

Total number of mutations - 13 (8 coding)

20 * coding mutations

- 17 -

Preferred variants of A/Sing/1/57/ca comprise the ones listed in the following Table 11, wherein "Δ" means "del" or "delta" and stands for a mutant that contains at least one "deletion" in its NS gene segment.

5 Table 11: Preferred variants of A/Sing/1/57/ca

	A/Sing/1/57/ca	Sing ca/ ΔNS 87	Sing ca/ ΔNSPR8	Sing ca/ NS124PR8
PB2 (Sing ca*)				
PB1 (Sing ca*)				
PA (Sing ca*)				
HA				
NP (Sing ca*)				
NA				
M1,2 (Sing ca*)				
NS1,2 (Sing ca*)				
NS1,2 (PR8**)				
Phenotypes				
ca	+	+	+	+
ts	+	+	+	+
IFN-induct.	-	- (?)	+	+
IFN-sensit	-	+	+	-

* genome segment originating from A/Singapore/1/57/ca

** genome segment originating from Influenza A/PR8/34

ca - cold adapted; ts - temperature sensitive;

aa - amino acid(s)

10 IFN-induct. - strain causes interferon release in host substrates that are able of IFN production, as well as in animal or human immune systems upon administration.

IFN-sensit. - strain is sensitive towards interferon; replication in IFN producing systems is reduced or stopped.

- 18 -

Sing ca/ΔNS 87 - strain A/Singapore/1/57/ca containing deletion of 87 amino acids in NS1 gene at aa position 36-123.

Sing ca/ΔNSPR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 (herein also abbreviated "PR8") which contains a deletion of of the entire NS1 gene.

Sing ca/NS124PR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 which contains a stop codon at aa position 124 of the NS1 gene.

- 10 The following Tables 12 and 13 refer to preferred Influenza B master strain candidates and to variations and reassortants, respectively, thereof.

Table 12: Full Sequence of the 8 genome segments and of the 11 corresponding proteins of strain B/Vienna/1/99/ca

B/Vienna/1/99/ca			
RNA segment	Nucleotide sequence. (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 19	PB2	SEQ ID No. 27
2	SEQ ID No. 20	PB1	SEQ ID No. 28
3	SEQ ID No. 21	PA	SEQ ID No. 29
4	SEQ ID No. 22	HA ₀	SEQ ID No. 30
5	SEQ ID No. 23	NP	SEQ ID No. 31
6	SEQ ID No. 24	NB	SEQ ID No. 32
		NA	SEQ ID No. 33
7	SEQ ID No. 25	M1	SEQ ID No. 34
		BM2	SEQ ID No. 35
8	SEQ ID No. 26	NS1	SEQ ID No. 36
		NS2	SEQ ID No. 37

15 ca - cold adapted

The original strain B/Vienna/1/99 was isolated on Vero cell culture grown with serum-free medium in February 1999 in Vienna, Austria from a 12 year old female with acute influenza. It was rated as B/Beijing/184/93-like by the Center for Disease Control (CDC), Atlanta, USA. After an additional passage at 33°C the wildtype strain - designated as B/Vienna/1/99 wt - was attenuated by 22 serial passages at 25°C using the same cell culture system. The plaque purification was done at 25°C for the first and at 33°C for the following four

- 18 -

round. The derived plaque purified clone was amplified and stored at -70°C , designated as B/Vienna/1/99 ca or short BV22. The identity as a B/Beijing/184/93-like virus was confirmed by HI-assay with standard anti-serum from NIBSC.

B

Table 13: Mutations in B/Vienna/1/99/ca (=BV22) compared to B/Vienna/1/99/wt (BVie) 1. passage on Vero/SF

Segment (length in nucleotides)	Nucleotides changed			Protein (length in amino acids)	Amino acids changed		
	Position	BVie	BV22		Position	BVie	BV22
1 (2396)	-	-	-	PB2 (770)	-	-	-
2 (2369)	594	T	C	PB1 (752)	-	-	-
3 (2305)	-	-	-	PA (726)	-	-	-
4 (1882)	457 1299 1595	G G G	A T A	HA ₀ (584)	142 422 521	A K G	T N E
5 (1844)	128 330	C T	T C	NP (560)	23 -	S -	F -
6 (1557)	- 823 1135	- G T	- A C	NB (100) NA (466)	- 257 381	- R I	- Q T
7 (1190)	- 831	- A	- G	M1 (248) BM2 (109)	- 21	- M	- V
8 (1097)	118 -	G -	A -	NS1 (281) NS2 (122)	25 -	A -	T -

Example 5: Vaccine safety and efficacy

10

The subsequent data confirm temperature sensitivity and vaccine safety for influenza vaccines manufactured according to the present invention, e.g., as described in Example 1.

15 Table 14: Antibody response of mice after one intranasal immunisation without narcosis

Viruses	Number of responders ¹	GMT ³	Protection after challenge ²
PR8/Sing ca -2/6	0/6	< 4	5/6
PR8/Sing ca -ΔNS	4/6	6.7	5/6
PR8-wt	5/6	16.0	5/6

- 20 -

- 1 - number of animals with positive HI titer > 1:4
 2 - number of animals without detectable virus in the lungs
 3 - Geometric mean titer of antibodies in serum

5 PR8wt - influenza strain A/PR/8/34 wildtype (H1N1), pathogenic for mice
 PR8/Sing ca-2/6 - is the reassortant between attenuated influenza strain
 A/Sing/1/57 ca and PR8 wt, containing 2 genes (HA and NA) from PR8wt
 virus and all other genes from A/Sing/1/57 ca.

10 PR8/Sing-ΔNS contains HA and NA genes from PR8wt, five genes from
 A/Sing/1/57 ca and the NS gene of PR8 origin lacking the NS1 coding
 sequence (NS1 deletion or knockout).

Table 15: Antibody response and protection of mice after intranasal
 immunisation with different variants of A/Singapore/1/57 virus (under
 narcosis)

Viruses	Responders ¹		GMT after two immunisa- tions	Protection after challenge ⁴
	1-st immuni- sation	2-nd immuni- sation		
A/Sing/1/57/wt va ²	9/9	9/9	103.9	9/9
A/Sing/1/57/ca ³	8/10	10/10	65.7	8/10
A/Sing /57/ΔNS 87	1/10	10/10	27.9	8/10

- 1 - number of animals with positive HI titer > 1:4
 2 - va- Vero-adapted
 3 - ca - cold-adapted
 4 - number of animals without detectable virus in the lungs

20

Table 16: Reproduction of wt, va and ca variants of A/Singapore/1/57 in mouse
 lungs^a

Viruses	Virus titer in mouse lungs post infection on day, PFU/ml ^b		
	2	4	6
A/Singapore/1/57/wt	1.6x10 ⁶	2.2x10 ⁵	1.4x10 ³
A/Singapore/1/57/wt va	2.5x10 ⁵	2.1x10 ⁵	1.0x10 ²
A/Singapore/1/57/ca	<10	<10	<10

- 21 -

- ^a Mice were infected i.n. with 50 μ l of virus fluid with a titer 1.0×10^6 PFU/ml.
^b PFU/ml of 10% tissue suspension, titrated on MDCK cells.

Table 17: Virulence of wt and ca variants of A/Singapore/1/57 virus for ferrets

Viruses	Number of animals with fever post infection on day		
	1	2	3
A/Singapore/1/57 wt	FFF	NNN	NNN
A/Singapore/1/57 ca	NNN	NNN	NNN

5

Rectal temperature of animals was recorded twice a day and characterized as follows:

N - normal temperature from 38.1°C to 39.9 °C

F - fever, more than 40.0°C.

- 10 Each group consisted of 3 animals, which were immunized i.n. under ether narcosis with 1 ml of virus fluid with a titer of 2×10^6 PFU/ml.

Table 18: Reproduction of 2/6 reassortant of A/Hong Kong/1035/98 wt and A/Singapore/1/57/ca in mouse lungs^a

Viruses	Virus titer in mouse lungs on day 2-6 post infection, PFU/ml ^b		
	2	4	6
A/Hong Kong/1035/98 wt H1N1	6.8×10^4	2.0×10^4	< 10
A/Singapore/1/57/ca x A/Hong Kong/1035/98 wt	< 10	< 10	< 10

15

^a Mice were infected i.n. under ether narcosis with 50 μ l of virus fluid.

^b PFU/ml of 10% tissue suspension, titrated on Vero/SF cells, data are given as mean value for 6 mice (the lungs of each animal were treated separately).

The reassortant contains the HA and NA genes from A/Hong Kong/1035/98 wt

- 20 wildtype and the other 6 genes from A/Singapore/1/57/ca.

- 22 -

Table 19: Virulence of 8/2 reassortant of A/Vienna/47/96 wt and A/Singapore/1/57/ ca for ferrets

Viruses	Virus subtype	Number of animals with fever on day			
		1	2	3	Rhinitis ^a
<i>Master strain</i> A/Singapore/1/57/ ca	H2N2	NNN	NNN	NNN	±
<i>Epidemic virus</i> A/Vienna/47/96 wt	H3N2	NNN	FFF	FFF	+++
<i>Reassortant</i> A/Singapore/1/57/ca x Vienna/47/ 96 wt	H3N2	NNN	NNN	NNN	±

Animals were immunized i.n. under ether narcosis with 1 ml of virus, 2×10^5 PFU/ml.

5 N- normal temperature from 38.1°C to 39.9°C;

F- fever, more than 40.0°C.

^b +++ - severe rhinitis

± absence of rhinitis

10 The results presented in Tables 16 to 19 clearly demonstrate the safety of the vaccines containing the attenuated, temperature sensitive master strain or, in case of reassortants, of the vaccines based on the reassorted viruses composed of the "backbone" of the attenuated, temperature sensitive master strain (6 genes) and the HA and NA genes from, e.g., the pathogenic wildtype strain

15 A/Hong Kong/1035/98 wt.

Table 20: Ts and ca phenotype of B/Vienna/1/99

Virus	PFU/ml on Vero cells at	PFU/ml on MDCK cells at	
	25°C	33°C	39°C
B/Vienna/1/99 wt	<300	4×10^6	4×10^5
B/Vienna/1/99 ca (BV22)	1×10^6	2.4×10^6	<20

- 23 -

Table 21: Genetic stability of the ts phenotype of B/Vienna/1/99 ca

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	4×10^8	4×10^8
B/Vienna/1/99 ca (BV22)	2.4×10^8	< 20
B/Vienna/1/99 ca (BV22) after 5 passages at 33°C	8×10^5	< 20

The strain BV22 was passaged five times at high MOI on Vero cells. Then the ts-phenotype was controlled again. The strain remained temperature sensitive as can be seen in Table 21.

5

Table 22: Virulence of B/Vienna/1/99 ca and wt in mouse lungs

Virus	organ	PFU/ml* at day post infection		
		2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	1×10^2	1×10^2	20
B/Vienna/1/99 wt	lung	8×10^4	7×10^3	4.4×10^3
	nose	3.8×10^4	3.4×10^4	1.4×10^4

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

10

The data show that moderate reproduction of the ca master strain candidate BV22 was possible in the nasal mucosa while the ts property of the virus prevented reproduction in the lungs.

15

Table 23: Ts and ca phenotype of the reassortant influenza B strain

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	4×10^8	4×10^8
B/USSR/69 wt	1.6×10^8	4×10^4
B/Vienna/1/99 ca (BV22)	1.4×10^8	< 20
BV22 x B/USSR/69 (6/2)	8×10^8	< 20

- 24 -

A 6/2 reassortant strain containing HA and NA of the wild type influenza strain B/USSR/69 wt and the other 6 genome segments from B/Vienna/1/99 ca (BV22) was established. The origin of the hemagglutinin was tested by HI-assay, all other genome segments by RT-PCT and restriction analysis using 5 methods known in the art.

Table 24: Virulence of the reassortant influenza B strain in mouse lungs

Virus	organ	PFU/ml* at day post infection		
		2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	< 20	1×10^2	40
B/USSR/69 wt	lung	1.8×10^5	4×10^5	2.4×10^4
	nose	1.6×10^5	2×10^5	1.6×10^5
BV22 x B/USSR/69 wt (6/2)	lung	< 20	< 20	< 20
	nose	2.8×10^3	2×10^3	4×10^2

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

Example 6: Clinical study

15 The following vaccines (in the form of nasal sprays) were produced according to the present invention (e.g. as described in Example 1) for intranasal delivery.

Composition per ml (after reconstitution of freeze-dried material):

- (1) Placebo: 2x SF-medium, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- 20 (2) Vero-Vac H1: A/Beijing/262/95 (H1N1)-like preparation comprising 4.3×10^7 TCID₅₀ of 6/2 reassortant A/Singapore/1/57/ca with A/Hong Kong/1035/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (3) Vero Vac H3: A/Sidney/5/97 (H3N2)-like preparation comprising 2.1×10^7 TCID₅₀ of 6/2 reassortant A/Singapore/1/57/ca with A/SW/7729/98; 2x
- 25 culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (4) Vero Vac B: B/Beijing/184/93 - like preparation comprising 3.8×10^7 TCID₅₀ of 7/1 reassortant B/Vienna/1/99/ca (all genome segments

- 25 -

except HA) with B/Switzerland/4291/97 (HA genome segment); 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

- (5) Vero Vac Trivalent: mixture of (2), (3), and (4):
- | | | | |
|---|--|---------------------|--------------------|
| 5 | A/Singapore/1/57/ca x A/Hong Kong/1035/984 | 4.3x10 ⁷ | TCID ₅₀ |
| | A/Singapore/1/57/ca x A/SW/7729/98 | 2.1x10 ⁷ | TCID ₅₀ |
| | B/Vienna/1/99/ca x B/Switzerland/4291/97 | 3.8x10 ⁷ | TCID ₅₀ |
- (6) Russian trivalent vaccine (live influenza vaccine for adults):
- | | | | |
|----|---------------------------|---------------------|-------------------|
| 10 | A/17/Beijing/95/25 (H1N1) | 1.1x10 ⁸ | EID ₅₀ |
| | A/17/Sidney/97/76 (H3N2) | 2.3x10 ⁷ | EID ₅₀ |
| | B/60/Petersburg/95/20 | 1.1x10 ⁷ | EID ₅₀ |
- (7) Monovalent Vero vaccine BV22: B/Beijing/184/93 - like preparation comprising 2x10⁶ TCID₅₀ of master strain candidate B/Vienna/1/99/ca (=BV22); 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- 15

The vaccines were administered to 13 volunteers per each vaccination group. 550 µl of reconstituted vaccine (or placebo, respectively) were given intranasally to each patient on day 0 and for a second time on day 22 ± 1. The results are summarized in Table 25 below.

20

Safety results: The total number of adverse events (AE) during five days after the first and second vaccination was 14 including 9 mild and 4 moderate AE. Only one volunteer showed severe AE, comprising an increase in body temperature up to 38.8°C within 3 hours after the first vaccination without any local or systemic symptoms. During the next four hours his temperature became normal again. After the first vaccination 7 AE were observed. One of them was local and six were systemic. After the second vaccination 2 local and 5 systemic AE were observed.

25

30

No significant difference in terms of safety was revealed between the groups of the study including the one with placebo. No serious AE related to the vaccination were observed except for the one mentioned above. Two of the moderate AE occurred in the H3N2 group (temperature elevation up to 37.6° and acute pharyngitis on day 3 in one volunteer; nasal obstruction, discomfort in the throat on day 22-24 and temperature elevation up to 37.5°C in another volunteer), and one in the H1N1 group (pain in the throat, rhinitis from day 22-26, temperature elevation up to 37 - 37.8°C between days 22-24).

35

- 26 -

Table 25: Response of seronegative volunteers to Vero Vac vaccines and to a trivalent Russian cold-adapted egg derived vaccine

No	Vaccine for immunization	Virus dose, TCID ₅₀ /ml or EID ₅₀ /ml	No. of volunteers	% of volunteers with at least 4-fold increase of serum HA1 antibody titre to antigens		
				H1N1	H3N2	B
1	Placebo		13		(8)	
2	Vero Vac H1 (H1N1)	4.3x10 ⁷	13	46		
3	Vero Vac H3 (H3N2)	2.1x10 ⁷	13		77	
4	Vero Vac B	3.8x10 ⁷	12			50
5	Vero Vac Trivalent	H1 4.3x10 ⁷ H3 2.1x10 ⁷ B 3.8x10 ⁷	12	33	50	33
6	Russian trivalent vaccine: A/17/Beljing/95/26 H1N1 A/17/Sidney/97/76 H3N2 B/60/Petersburg/95/20	 1.1x10 ⁸ 2.3x10 ⁷ 1.1x10 ⁷	13	46	8	38
7	Vero vaccine BV22	2x10 ⁶	13			

The results obtained from the clinical study thus confirm a very good safety of
 5 the vaccines produced according to the present invention and using the
 preferred influenza A and B master strain candidates of the present invention.

- 27 -

CLAIMS

We claim

1. A process for multiplying viruses in a host cell system wherein alterations
5 in the surface antigens of the starting virus due to adaptive selection on said
host cell system are minimized or prevented, the process comprising the steps
of:
a) Infecting African Green Monkey Kidney (Vero) cells that have been grown
in and separated from a suitable serum-free medium that does not contain
10 functional proteins such as insulin, transferrin or growth factors, by adding to
the cells a quantity of a virus suspension and incubating the cells for a period of
time;
b) after incubation removing the virus suspension from the cells and adding
to the cells a quantity of a suitable serum-free medium that does not contain
15 functional proteins such as insulin, transferrin or growth factors and that has
been supplemented with human recombinant or porcine trypsin and/or
trypsinogen; and
c) incubating the cells for another period of time and harvesting the
multiplied viruses by collecting the supernatant obtained from centrifugation of
20 the resulting cell culture.
2. The process according to claim 1, wherein the medium in step (b) has
been further supplemented with a nuclease enzyme having DNase and/or
RNAse activity, preferably with Benzonase.
- 25 3. The process according to claim 1 or 2, wherein the medium in step (b)
has been supplemented once with trypsin in a concentration of 0.5 - 10,
preferably 2 - 5 µg per ml medium.
- 30 4. The process according to any one of claims 1 to 3, wherein the period of
time for incubation in step (a) ranges from 10 - 120 minutes, preferably from 30
- 60 minutes.
5. The process according to any one of claims 1 to 4, wherein the period of
35 time for incubation in step (c) ranges from 24 - 96 hours, preferably from 48 -
72 hours.

- 28 -

8. The process according to any one of claims 2 to 5, wherein the medium has been supplemented once with Benzonase in an amount of 2 - 30, preferably 5 - 15, U per ml of medium.
- 5 7. The process according to any one of claims 1 to 6, wherein the virus is an Influenza A virus, preferably of subtype H3N2, or Influenza B virus.
8. The process according to claim 7, wherein the virus is an attenuated, cold adapted, temperature sensitive influenza virus selected from the group
- 10 consisting of A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99 ca, and any attenuated variants and reassortants derived from these strains.
9. An influenza vaccine obtainable in a process as defined in any one of
- 15 claims 1 to 8, in combination with a suitable carrier.
10. The vaccine according to claim 9, for intranasal delivery.
11. An attenuated, cold adapted, temperature sensitive influenza virus
- 20 selected from the group consisting of influenza virus strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, and B/Vienna/1/99 ca, and any attenuated variants and reassortants derived from these strains.
- 25 12. The influenza virus according to claim 11, obtainable in a process as defined in any one of claims 1 to 8.
- 13 A vaccine comprising at least one influenza virus defined in claim 12, in combination with a suitable carrier.
- 30 14. The vaccine according to claim 13 for intranasal delivery.
15. The vaccine according to claim 13 or 14, obtainable in a process according to any one of claims 1 to 8.
- 35

- 29 -

ABSTRACT

The invention relates to a simple and efficient process for isolating viruses from various sources and for producing live attenuated influenza vaccines in a serum-free Vero cell culture under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or prevented. The process does not require purification of the virus-containing supernatant harvested from the cell culture nor post-incubation treatment of the viruses for HA activation.

- 10 The invention further relates to influenza A and B master strain candidates and to vaccines made thereof.

Fig.1

1/1

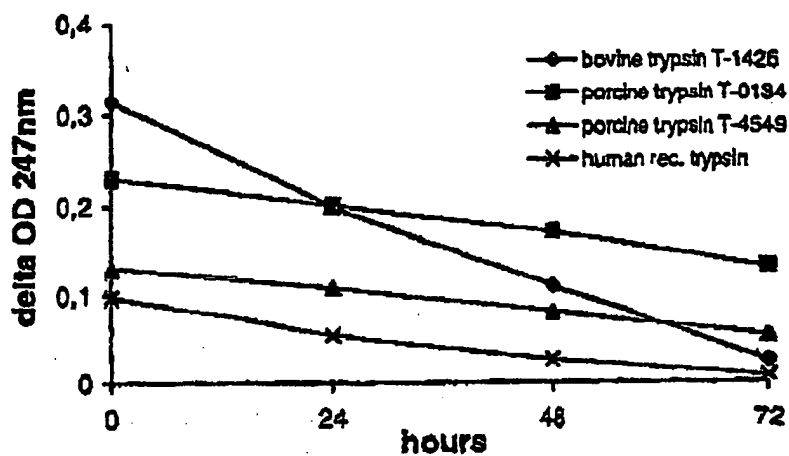


Fig. 1

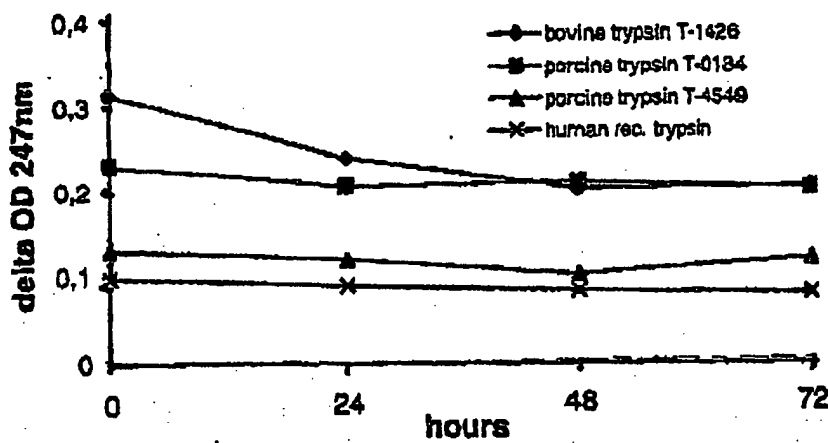


Fig. 2

interbcs 2-2000

SPEC

00720896

SEQUENCE LISTING

<110> Polymun Scientific Immunbiologische Forschung GmbH

<120> INFLUENZA VACCINE AND METHOD OF MANUFACTURE

<130> BEP-4847-EU (Influenza vaccine)

<140>

<141>

<160> 37

<170> PatentIn Ver. 2.1

<210> 1

<211> 2341

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 1

```

agcaasagca ggtcaattat attcaatatg gaaagaataa aagaactacg gaatctgatg 60
tcgcagtcctc gcaotcgcga gatactaaca aaaaccacag tggaccatat ggccataatt 120
aagaagtaca catcagggag acaggaaaag aaccctgcac ttaggatgaa atggatgatg 180
gcaatgaaat atccgattac agctgacaaag aggataacag aaatgatccc tgagagaaat 240
gagcaagggc agactctatg gagtaaatg aatgatgccg gatcggatcg agtgaaggta 300
tcacctctgg ctgtgacatg gtggaataga aatggacca aacaaagtac gggtcattat 360
ccaaaatctc acaaaaacta ttttgagaaa gtccgaaggt taaaacatgg aacctttggc 420
cotgtocatt ttagaanaaa agtcaaaaata cggccgaagag ttgcataaaa tcotgggtcat 480
gcagacotca gtgccaagga ggacacagat gtaatcatgg aagtgtttt cctaacgaa 540
gtgggggcca ggatactaac gtccgaatcg caattaacaa caaccnaaga gnaaaaagaa 600
gaactccagg attgcaaat tctccrttg atgggttgoat acatgttaga gagagaactt 660
gtccgaaaaa cagagattct cccagttgct ggtggaacaa gcagtgtgta cattgaagt 720
ttgcaacttae ctcaaggaa atgctgggaa cagatgtaca ctccaggtgg agaagtgaag 780
aatgatgatg ttgatcaag tctaattatt gcagccagga acatagtga aagagcagca 840
gtatcagcag atccactagc atctttattg gagatgtgcc acagcacaca gattggcggg 900
scaaggatgg tggacattct taggcagaa ccaacgggag agcaagctgt ggatatatgc 960
aagggtgcaa tgggactgag aatcagctca tcttcagtt ttggcgggtt cacatttaag 1020
agaacaagcg gatcatcagt caagatagag gaagaagtgc ttacgggcaa tcttcaaaa 1080
ttgaaaataa ggggtgcatg gggatcagag gagttcaca ttgttgggaa aagggaaca 1140
gctatactca gaaagcaac caggagattg attcagctga tagtgagtgg aagagacgaa 1200
cagtcgatag ccgaagcaat aattgtggcc atggattttt cacaagaaga ttgtatgata 1260
aaagcagtta gaggtgact gaatttogg aatagggcaa atcagcogtt gaatcccatg 1320
catcaacttt taagacattt tcagaaggat gcgaaagtgc tttttcaaaa ttggggaatt 1380
gaacatctcg acaatgtgat gggatgatt ggggtattac cagacatgac tocaagaca 1440
gagatgtcaa tgagaggggt aagagtcagc aaaatgggag tagatgasta ctccagcgg 1500
gagagagtag tggtagcat tgaccggtt ttgagagttc gagaccaacg aggaantgta 1560
ctactatctc ctgaggaggt cagtgaacaa cagggaacag aaaaactgac aetaacttac 1620

```

tcacgtcaca tgatgtggga gattaatggc octgagtcag tgttgggtcaa tacctatcag 1680
 tggatcatca gaaactggga aactgttaa attcagtggt ctcaaatcc tacaatgcta 1740
 tacaataaaa tggaaattga gccatttcag totttagttc ctaaggccat tagaggccaa 1800
 tacagtgggt ttgttaggac tctattccaa caaatgaggg atgtacttgg gacatttgat 1860
 accaccaga taataaaact tcttcccttt gcagccggcc caccaaaagca aagtagaatg 1920
 cagttctctt cattgactgt gaatgtgagg ggatcaggaa tgagaatact tgttaaggggc 1980
 aattctctctg tattcaacta caacaagacc actaagagac taacaattct cggaaaggat 2040
 gctggcactt taactgaaga cccagatgaa ggcacatctg gagtggagtc cgtgttctg 2100
 agaggattcc tcattctggg caaagaagat agggatatg gaccagcatt aagcatcaat 2160
 gaactgagta accttgcgaa aggagaaaag gctaatgtac taattgggca aggagcgtg 2220
 gtgttggtaa tgaacgaaa acgggactct agcatactta ctgacagcca gacagcgacc 2280
 aaagaattc ggatggccat caattaatgt tgaatagtt aaaaacgacc ttgtttctac 2340
 t 2341

<210> 2

<211> 2341

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 2

agcaaaagaa ggaaccat ttgaatggat gtcaatccga ccttactttt cttgaaagtt 60
 ccagcgcaaa atgccataag tactacattc ccttatactg gagatccctc atacagccat 120
 ggaacaggaa caggatacao catggacaca gtcaacagaa cacatcaata ttcagaaaag 180
 gggaaagtga caacaaacac ggaanctgga gcgcccaac ttaacccaat tgatggacca 240
 ctacctgagg acaatgaacc aagtggatat gcacaaacag actgcgtect ggaagcaatg 300
 gcttcccttg aagaatccca cccgggaatc ttgaaaact cgtgtcttga aacgatggaa 360
 gttattcaac aaacaagagt ggacaaactg acccaaggtc gtcagacctc tgattggaca 420
 ttgaacagaa atcagccggc tgcaactgag ctgccaaca ctatagaggt cttoagatcg 480
 aatggctctg cagctaatga atcgggaagg ctaatagatt tcttcaagga tgtgatgaa 540
 tcaatggata aagaggagat ggaataaca acacacttcc aaagaaaag aagagtaag 600
 gacaacatga ccaagaanaa ggtcacacaa cgaacaatag gaaaaaagaa gcaagagattg 660
 aacaagagaa gctatctaat aagagcaatg acattgaaca caatgactaa agatgcagag 720
 agaggttaat taagagaag agcaattgca acacccggtc tgcagatoag agggctcgtg 780
 tactttgtcg aaacactagc gagaagtatt tgtgagaagc ttgaacagtc tgggtctccg 840
 gttggaggta atgaaaagaa ggctaaactg gcaaatgttg tgagaaaaat gatgactaat 900
 tcacaagaca cagagctctc ttccacaatt actggagaca ataccaaatg gaatgagaat 960
 caaaatcttc ggatgttctc ggcgatgata acatcacatc caagaaatca acctgaatgg 1020
 tttagaaaag tcttgagcat cgcacctata atgttctcaa ataaaatggc aagactaggg 1080
 aaaggatata tgttcgaaag caagagcatg aagctccgaa cacaataacc agcagaaatg 1140
 ctagcaagta ttgacctgaa atactttaat gaatcaacaa gaaagaaaat cagagaaata 1200
 aggcctctcc taatgatagg cacagtctca ttgagtcctg gaatgatgat gggcatgttc 1260
 aacatgctaa gtacagtcac aggagtctca atcctgaatc ttggscaaaa gaagtacacc 1320
 aadacaacat actggtggga cggactccaa tctctgatg acttcgcctc catagtgaat 1380
 gcaccaaatc atgagggaaat acaagcagga gtggatagat tctacagaac ctgnaagcta 1440
 gtcggaatca atatgagcaa aaagaagtc tacataaata ggcagggac atttgaatro 1500
 acaagctttt tctatcgcta tggatttcta gccaattta gcattggagct gccagtttt 1560
 ggagtgtctg gaattaatga atcggctgat atgagcattg gggtaacagt gatnaagaa 1620
 aacatgataa acaatgacct tgggccagca aagcccaaa tggctcttca actattcatc 1680

1119404 12-2001

SPEC

0012089

AAAGACTACA GATATACGTA CCGGTGCCAO AGAGGAGACA CACAAATTCA GACAAGGAGA 1740
 TCATTGAGC TAAAGAAGCT GTGGGAGCAA ACCCGTCAA AGGAGGACT TTTGGTTTCG 1800
 GATGGAGGAC CAAACTTATA CAATATCCGG AATCTCCACA TTCCAGAGT CTGCTGAAG 1860
 TGGGAGCTAA TGGATGAAGA CTATCAGGGG AGGCTTTGTA ATCCCTGAA TCCATTCTC 1920
 AGTCATAAGG AGATTGAGTC TGTAACAAT GCTGTGGTAA TGCCGCTCA CGGTCCAGCC 1980
 AAGAGCATGG AATATGATGO TGTCGTACT ACACACTCCT GGATCCCTAA GAGGAACCGO 2040
 TCCATTOTCA ACACAAGCCA AAGGGGAATT CTTGAGGATG AACAGATGTA TCAGAAGTGT 2100
 TGCAATCTAT TCGAGAAATT CTCCCTAGC AGTTCGTACA GGAGACCAGT TGGAAATTCO 2160
 AGCATGCTGG AGGCCATGGT GTCTAGGGCC CGGATTGATG CACGGATTGA CTTGAGTCT 2220
 GGACGGATTA AGAAAGAGGA GTTCGTGAG ATCATGAAGA TCTGTTCCAC CATTGAAGAG 2280
 CTCAGACGGC AAAAAATAGT AATTAGCTT GTCCTTCATG AAAAAATGCC TTGTTCTAC 2340
 T 2341

<210> 3

<211> 2233

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 3

AGCAAAAGCA GGTACTGATC CGAAATGGAA GATTTTGTGC GACAATGOTT CAATCCGATG 60
 ATTGTGAGC TTGCGGAAAG GGCAATGAAA GAGTATGGAG AGGATCTGAA AATCGAAACA 120
 AACAAATTG CAGCAATATG CACTCACTG GAAGTATGCT TCATGTATTC AGATTTTCAT 180
 TTCATCAATG AGCAAGGCGA GTCAATAATA GTAGAGCTTG ATGATCCAAA TGCACTTTTG 240
 AAGCACAGAT TTGAATAAT AGGGGGAAGA GATCGOACAA TGGCOTGGAC AGTAGTAAAC 300
 AGTATTTGCA ACCTACAGG AGCTGAGAAA CCGAAGTTT TGCAGATTT GTATGATTAC 360
 AAGGAGAATA GATTCATCGA GATTGGAGTG ACAAGGAGGG AAGTCCACAT ATACTATCTT 420
 GAAAAGGCCA ATAAATTAA ATCTGAGAAG ACACACATCC ACATTTCTCT ATTCCTGGG 480
 GAAGAAATGG CCACAAAGGC CGACTACACT CTCGATGAGG AAAGCAGGGC TAGGATCAAA 540
 ACCAGACTAT TCACCATAAG ACAAGGAATG GCTAGCAGAG GCCTCTGGGA TTCTTTCTGT 600
 CAGTCCGAAA GGGCGAAGA AACCAATTGA GAAAGATTG AAATCAGG GACAATGCGC 660
 AGGCTCGCG ACCAAAGTCT CCGCGGAAAC TTCTCTGCGC TTGAGATTT TAGAGCCTAT 720
 GTGATGGAT TCGAAOCGAA CGGTACATT GAGGGCAAGC TTTCTCAAT GTCCAAAGAA 780
 GTAATGCTA AAATTGAACC TTTCTGAAA ACAACACCAA GACCAATTAG ACTTCGGAT 840
 GGGCTCTCT GTCTCAGCG GTCCAAATTC CTGCTGATGG ATGOTTAAA ACTAAGCATT 900
 GAGGACCCAA GTOACGAAGG AGAGGGAATA CCCTATATG ATGCGATCAA GTGTATGAGA 960
 ACATTCTTTG GATGGAAGA ACCCTATGTT GTAAACCAAC ACGAAAAGGG AATAAATCCA 1020
 AATTATCTGC TGTCAATGAA GCAAGTACTG GCAGAACTGC AGGACATTGA GAATGAGGAG 1080
 AAGATTCCAA GAACCAAAAA CATGAAGAAA ACGAGTCAGC TAAAGTGGGC ACTTGGTGAG 1140
 AACATGGCAC CAGAGAAGGT AGACTTTGAC GACTGTAGAG ATATAAGCGA TTTGAAGCAA 1200
 TATGATAGTG ATGAACCTGA ATTAAGGTCA CTTTCAAGCT GGATCCAGAA TGAGTTCAAC 1260
 AAGGATGCG AGCTGACCAA TTCAATCTGG ATAGAGCTCG ATGAGATTGG AGAAGATGTG 1320
 GCTCCAATTG AACACATTGC AAGCATGAGA AGGAATTACT TCACAGCAGA GGTGTCTCAT 1380
 TGCAGAGCCA CAGAATATAT AATGAAGGG GTATACATTA ATACAGCCTT GCTTAATGCA 1440
 TCTGTGCGAG CAATGGACGA TTTCCAACTA ATTCCATGA TAAGCAAGT TAGAAGTAAA 1500
 GAGGGAAGGC GAAAGACCAA TTTATATGTT TCCATGTA AAGGAAGATC TCACTTAAGG 1560
 AATGACACCG ACGTGGTAAA CTTTGTGAGO ATGGAGTTT CTCTCACTGA CCCAAGACTT 1620
 GGGCCAOACA AATGGGAGAA GTACTGTGTC CTTGAGATAG GAGATATGCT ACTAAGAAGT 1680
 GCCATAGGCC AGGTGTCAAG GCCATGTTT TTGTATGTA GGACAAATGG AACATCAAG 1740

```

attaaaatga aatggggaat ggagatgagg cgttcgcctcc ttcagtcact ccaacaaatc 1800
gagagoatga ttgaagocca gtccctctgc aaggagaaag acatgaccaa agagtttttc 1860
gagaataaat cagaacatg gcccttcgga gagtccocta aaggagtggg agaagggtcc 1920
attgggaagg totgcaggac ttattatgac aagtcgggtat tccatagcct gtatgcattc 1980
ccacaattag aaggattttc agctgaatca agaaaactgc tccttctcgt tcaggctctt 2040
agggacaatc ttgaacctgg gacctttgat cttgqggggc tatatgaagc aattgaggag 2100
tgcctgatta atgatccctg ggttttgctt aatgcgcttt ggttcaactc cttcctaaca 2160
catgcattaa gatagtgtg gcaatgctac tatttgctat ccatactgtc caaaaagta 2220
ccttgtttct act 2233

```

<210> 4

<211> 1773

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 4

```

agcaaaagca ggggttatac catagacaac cagaagcaaa acaatggcca tcatttatct 60
cattctctcg ttcacagcag tgagagggga ccagatatgc attggatacc atgccaatag 120
ttccacagag aaggtogaca caattctaga gcagacgcto actgtgactc atgccaaagg 180
cattcttgag aagaccata acggaaagtt atgcaacta aacggaatcc ctccacttga 240
actaggggac tgtagcattg ccggatggct ccttggaat ccagaatgtg ataggcttct 300
aagtgtgcca gaatggtccr atataatgga gaaagaaaac ccgagagacg gtttgtgta 360
tccaggcagc ttcaatgatt atgaagaatt gaaacatctc ctcagcagcg tgaacattt 420
cgagaaagta aagattctgc ccaagatag atggacacag catacaacaa ctggagggtc 480
acgggctctg cgggtgtctg gtaatccatc attcttcagg aacatggtct ggctgacaaa 540
gaaagaatca aatratcogg ttgccaagg atcgtacaa aatacaagcg gagaacaaat 600
gctaataatt tggggggtgc accatcccaa tgatgagaca gaacaaagaa cattgtacca 660
gaatgtggga acctatgttt ccgtaggcac atcaacattg aacaaaaggt caacoccaga 720
catagccaca aggcctaaag tgaatggact aggaagtga atggaattct cttggaacct 780
attggatatg tgggacacca taatttttgc gactactggt aatctaattg caccagagta 840
tggattcaaa atatcgaaaa gaggtcaattc agggatcatg aaaacagaag gaacacttga 900
gaactgtgag accaatgccc aaactccttt gggagcaata aatacaacat tgccttttca 960
caatgtccac ccactgacaa taggtgagtg ccccaaatat gtaaaatcgg agaagttggt 1020
cttagcaaca ggaccaagg atgttccca gattgaatca agaggattgt ttgggggaat 1080
agctgggttt atagaaggag gatggcaagg aatggttgat ggttggtatg gataccatca 1140
cagcaantgac cagggatcag ggtatgcagc agacaaagaa tccactcaaa aggcatttga 1200
tggaatcacc aacaaggtea attctgtgat tgaaaagatg aacaoccaat ttgaagctgt 1260
tgggaaagaa ttcagtaact tagagagaag actggagaac ttgaacaaa agatggaaag 1320
cgggtttcta gatgtgtgga catacaatgc tgagcttcta gttctgatgg aaatgagag 1380
gacacttgac tttcatgatt ctaatgtcaa gaatctgtat gataaagtca gaatgcagct 1440
gagagacaac gtcaaaagac taggaatgg atgttttga ttttatcaca aatgtgtga 1500
tgaatgcatt aatagtgtga aagacgggac gtatgattat cccaagtatg aagaagagtc 1560
taaaactaat agaaatgaaa tcaaaaggggt aaaattgagc agoatggggg tttatcaaat 1620
ccttgccatt tatgtacag tagcagggtc tctgtcactg gcaatcatga tggctgggat 1680
ctctttctgg atgtgtcca acgggtctct gcagtgcagg atctgcatat gattataagt 1740
cattttatca ttaaaaaaac cettgtttct act 1773

```

<210> 5

09-12-2000

SPEC

09-12-2000

<211> 1363
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 5
agcaaaagca gggtagataa toactcactg agtgacatca aaatcatggc gtcccaaggc 60
accaaacggg cttatgaaca gatggaact gatggggaac gccagaatgc aactgaatc 120
agagcatcog tggggaagat gattgatgga attggacgat tctacatcca aatgtgcacc 180
gaacttaaac tcagtgatta tgaggggcga ctgatccaga acagcttaac aatagagaga 240
atgggtctct ctgcttttga cgagaggagg aataaatato tggagaaca tcccagcggc 300
gggaaggatc ctaagaaac tggaggacc -atatacaaga gagtaaatgg aaagtggatg 360
agggaaactog tcttttatga caaagaaga ataggcgaa tctggcgcca agctaataat 420
ggtgatgatg caacagctgg tctgactcac atgatgatot ggcattccaa ttggaatgat 480
acaaotatcc agaggacaag agctottgtt cgcaccgga tggatccag gatgctct 540
ttgatgcagg gttcgcctot ccctaggagg tctggagccg caggcgctgc agtcaazaga 600
gttgggacaa tgggtgatga gttgatcagg atgatcaaac gtgggatcaa tgatcggaac 660
ttctggagag gtgagaatgg gcggaaaaa aggattgctt atgagagaat gtgcaacatt 720
ttcaaaaggaa aatttcaaac agctgoacaa agagcaatga tggatcaagt gagagaagc 780
cggaaaccag gaaatgctga gatcgaagat ctcatcttlo tggcacggtc tgoactata 840
ttgagagggt cagttgctca caaatcttgt ctgcttgcot gtggtatgg aactgcctga 900
gccagtgggt aogaactcga aaaagaggga tactctttag taggataga cctttcaaa 960
ctgcttcaaa acagocaaat atacagccta atcagacoga acgagaatcc agcacacaag 1020
agtcaagtgg tgtggatggc atgcaattct gctgcatttg aagatctaag agtatcaagc 1080
ttcatcagag ggaccaaagt aatccaaagg gggaaacttt ccactagagg agtacaatt 1140
gcttcaaatg aaacatgga tactatggaa tcaagtaact ttgaactgag aagcaggtag 1200
tgggocataa ggaccagaag tggaggaaac actaatcaac agagggcctc tgcaggtcaa 1260
atcagtgtac aacctacgtt ttctgtgcaa agaaccctcc catttgacaa aacaaccatc 1320
atggcagcat tcactgggaa tgcajaggga agaacatcag acatgagggc agaaatcata 1380
aggatgatgg aaggtgaaa accagaagaa gtgtocctcc agggcgggg agtcttcag 1440
ctctcggaag aaaaggcaac gaacccgatc gtgccctctt ttgacargag taatgaagga 1500
tcttatttct tggagacaa tgcagaggag tacgacaatt aaggaaaaat acccttgttt 1560
ctact 1563

<210> 6
<211> 1466
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 6
agcaaaagca ggagtgaaga tgaatccaaa tcaaaagata ataacaattg gctctgtctc 60
tctcaccatt gcaacagtat gcttctoat gcagattgcc atcotggaa ctactgtgac 120
attgcatttt aaacaacatg agtgcgactc ccccgcgagc aaccaagtta tgcattgtga 180
accaataata atagaaagga acataacaga gatagtgtat ttgaataaca ccaccataga 240
gaaagagatt tgcgccgaag tagtggata cagaaattgg tcaaaagcgc aatgtcaaat 300
tacaggattt gcaacctttt otaaggacaa ttcaatccgg ctttctgctg gtggggacat 360
ttgggtgacg agagaacctt atgtgtcatg cgatccctggc aagtgttata aatttgact 420
cgggcagggg accacactat acaacaaca ttcaaattggc acaatacarg atagaatccc 480
tcacgzaacc ctattaatga atgagttggg tgttcoattt catttaggaa ccaaacagt 540

Printed on 22-2009

STEC

06-208

```

gtgtgttagca tgggtccagct aaagtgtgtca cgtatgaaaa gcatgggttc atgtttgtgt 600
cactggggat gatagaaatg cgaactgctag ctctatttat gacgggaggc ttgtggacag 660
tattgggtca tgggtctcaaa atatccctcag gaocccaggag tcggaatgcg tttgtatona 720
tgggacttgc acagttagta tgaactgatgg aaagtcatca ggaagagccg atactagaat 780
actattcatt aaagagggga aaattgtccg tattagccca ttgtcaggaa gtgctcagca 840
tatagaggag tgttcctgtt accctcgata tctgaactgc agatgtatct gcagagacaa 900
ctggaaaggc tctaataaggc cgttataga cataaatatg gaagattata gcattgattc 960
cagttatgtg tgtcagggc ttgtggcga cacaccagg aacgaagaca gctctagcaa 1020
tagcaattgc agggatccta acaatgagag agggatcca gtagtgaaag gctgggcctt 1080
tgacaatgga gatgatgtat ggtgggag aacaatcaac aaagattcac gctcaggtta 1140
tgaaactttc aaagtcattg gtggttggtc caaacctaat tccaaatcgc aggtcaatag 1200
acaggtcata gttgacacaa ataattggtc tggttactct ggtattttct ctgttgaggg 1260
caaaagctgc atcaataggt gcttttatgt ggagttgata aggggaaggc cacaggagac 1320
tagagtatgg tggacctcaa acagtattgt tgtgttttgt ggcacttcag gtacttatgg 1380
aacaggctca tggcctgatg gggcgaaacat caatttcatt cctatataag ctttcgcaat 1440
tttagaaaaa actccttgtt tctact
1466

```

<210> 7

<211> 1027

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 7

```

agcaaaagca ggtagatatt gaaagatgag tcttotaacc gaggtcgaaa cgtatgttct 60
ctctatcgtc cgtcaggcc cctcaaaagc cgaatogaa aagagacttg aagatgtott 120
tgttgggag aaacaccgac ttgaggctct catggaatgg cttaaagacaa gaccaatcct 180
gtcacctctg actaagggga ttttgggatt tgtattcacg ctcaacctgc ccagtgagcg 240
aggactgcag cgtagacgct ttgtccaaaa tgccctcaat gggaaatggg atocaaataa 300
catggacaga gcagttaaac tgtataaaaa gcttaagagg gagataacat tccatggggc 360
caaaagaaata gogctcagtt attotgctgg tgcacttgcc agttgtatgg gcctcatata 420
caacaggatg ggggctgtga ccaactgaag ggcctttggc ctggtatgtg caacctgtga 480
acagattgct gactcccacc atagggtotca taggaaatg gtgaacacaa ccaatccact 540
aataagacat gagaacagaa tggttctggc cagcaetaca gctaaggcta tggagcaaat 600
ggctggatcg agtgagcaag cagcagaggc catggaaggt gctagtcagg coaggcaaat 660
ggtgcaggga atgagagcca ttgggactca tctagctcc agtgctggtc taaaagatga 720
tcttottgaa aatttgacag cctatcagaa acgaatgggg gtgcagatgc aacgattcaa 780
gtgacctctt tgttgtgoc gcagtatca ttgggatctt gcacttgata ttgtggatc 840
ttgatcgtc ttttttcaaa tgcatttctc gcttctttaa acacgggtgtg aaaagagggc 900
cttctacgga aggagtacca ggtotatga gggagaata tcgaaggaa cagcagagtg 960
atgtggatgc tgacgatagt catttgtca gcatagagct ggagtaaaaa actaccttgt 1020
ttctact
1027

```

<210> 8

<211> 890

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 8

Printed 03-12-2000

SPEC

00120898

agcaaaagca gggtagacaaa gacataatgg atcctaacac tgtgtcaagc tttcaggtag 60
attgcttctt ttggcatgtc cgcacaacag ttgcagacca agaactaggt gatgccccat 120
tcttgatcg gcttgcgcga gatcagaagt cctaagggg aagaggcago sctctoggtc 180
tgaacatcga aacagccacc cgtgttgga agcagatagt ggagaggatt ctgaagggaag 240
aatccgatga ggcacttaaa atgaccatgg cctcgcacc tgcttcgcga tacctaactg 300
acatgactat tgaggaaatg tcaagggact ggttcagct aatgcccaag cagaaagtgt 360
caggccctct ttgtatcaga atggaccagg caatcatgga taagaacatc atattgaag 420
cgaatttcag tgtgattttt gaccggctag agaccctaatt attactaagg gctttcaccg 480
aagaggggagc aattgttggc gaattttcac cattgccttc tcttcacgga cataactaag 540
aggatgtcaa aaatgcaatt ggggtcctca tcggaggact tgaatggaat gataacacag 600
ttcagagtctc taaactcta cagagattog cttggagaaa cagtaatgag aatgggagac 660
ctccactcac tccaaaacag aaacggaaaa tggcgagaac aattgggtca aaagttcgaa 720
gaataagat ggctgattga agaagtga cacaattga agataacaga gaatagtttt 780
gagcaaataa catttatgca agccttacag ctgctatttg aagtggaaac agagataaga 840
actttctcgt ttcagcttat ttaatgataa aaaacacct tgtttctact 890

<210> 9

<211> 771

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 9

Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
1 5 10 15

Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
20 25 30

Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
35 40 45

Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
50 55 60

Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
65 70 75 80

Met Asn Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val
85 90 95

Thr Trp Trp Asn Arg Asn Gly Pro Met Thr Ser Thr Val His Tyr Pro
100 105 110

Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly
115 120 125

Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg
130 135 140

Printed: 01-12-2001

SPEC

001208

Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln
 145 150 155 160
 Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile
 165 170 175
 Leu Thr Ser Glu Ser Gln Leu Thr Thr Thr Lys Glu Lys Lys Glu Glu
 180 185 190
 Leu Gln Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu
 195 200 205
 Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr
 210 215 220
 Ser Ser Val Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp
 225 230 235 240
 Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp
 245 250 255
 Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val
 260 265 270
 Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln
 275 280 285
 Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu
 290 295 300
 Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser
 305 310 315 320
 Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser
 325 330 335
 Ser Val Lys Ile Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu
 340 345 350
 Lys Ile Arg Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys
 355 360 365
 Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu
 370 375 380
 Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Ala Glu Ala Ile Ile Val
 385 390 395 400

03-12-2001

SPEC

00120896

Ala Met Val Phe Ser Gln Glu Asp Cys Met Ile Lys Ala Val Arg Gly
405 410 415

Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His
420 425 430

Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn
435 440 445

Trp Gly Ile Glu His Ile Asp Asn Val Met Gly Met Ile Gly Val Leu
450 455 460

Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val
465 470 475 480

Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Ala Glu Arg Val Val Val
485 490 495

Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu
500 505 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr
515 520 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser
530 535 540

Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val
545 550 555 560

Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu
565 570 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr
580 585 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly
595 600 605

Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
610 615 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val
625 630 635 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe
645 650 655

Printed 03-12-2001

SPEC

001208

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala
660 665 670

Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser
675 680 685

Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr
690 695 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu
705 710 715 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys
725 730 735

Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys
740 745 750

Arg Ile Arg Met Ala Ile Asn Xaa Cys Xaa Ile Val Xaa Lys Arg Pro
755 760 765

Cys Phe Tyr
770

<210> 10
<211> 757
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 10
Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn
1 5 10 15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
20 25 30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
35 40 45

Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro
50 55 60

Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65 70 75 80

Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu

00120896

SPEC

00120896

85	90	95
Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu		
100	105	110
Val Ile Gln Gln Thr Arg Val Asp Lys Leu Thr Gln Gly Arg Gln Thr		
115	120	125
Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala		
130	135	140
Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser		
145	150	155
Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Ile Glu Ser Met Asp Lys		
165	170	175
Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg		
180	185	190
Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys		
195	200	205
Lys Gln Arg Leu Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu		
210	215	220
Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala		
225	230	235
Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu		
245	250	255
Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro		
260	265	270
Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys		
275	280	285
Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly		
290	295	300
Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala		
305	310	315
Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val		
325	330	335
Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly		

Printed 08-12-2000

SPED

001200

340	345	350
Lys Gly Tyr Met Phe Glu Ser	Lys Ser Met Lys Leu Arg Thr Gln Ile	
355	360	365
Pro Ala Glu Met Leu Ala Ser	Ile Asp Leu Lys Tyr Phe Asn Glu Ser	
370	375	380
Thr Arg Lys Lys Ile Glu Lys	Ile Arg Pro Leu Leu Ile Asp Gly Thr	
385	390	395
Val Ser Leu Ser Pro Gly Met	Met Met Gly Met Phe Asn Met Leu Ser	
405	410	415
Thr Val Ile Gly Val Ser Ile	Leu Asn Leu Gly Gln Lys Lys Tyr Thr	
420	425	430
Lys Thr Thr Tyr Trp Trp Asp	Gly Leu Gln Ser Ser Asp Asp Phe Ala	
435	440	445
Leu Ile Val Asn Ala Pro Asn	His Glu Gly Ile Gln Ala Gly Val Asp	
450	455	460
Arg Phe Tyr Arg Thr Cys Lys	Leu Val Gly Ile Asn Met Ser Lys Lys	
465	470	475
Lys Ser Tyr Ile Asn Arg Thr	Gly Thr Phe Gln Phe Thr Ser Phe Phe	
485	490	495
Tyr Arg Tyr Gly Phe Val Ala	Asn Phe Ser Met Glu Leu Pro Ser Phe	
500	505	510
Gly Val Ser Gly Ile Asn Glu	Ser Ala Asp Met Ser Ile Gly Val Thr	
515	520	525
Val Ile Lys Asn Asn Met Ile	Asn Asn Asp Leu Gly Pro Ala Thr Ala	
530	535	540
Gln Met Ala Leu Gln Leu Phe	Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg	
545	550	555
Cys His Arg Gly Asp Thr Gln	Ile Gln Thr Arg Arg Ser Phe Glu Leu	
565	570	575
Lys Lys Leu Trp Glu Gln Thr	Arg Ser Lys Ala Gly Leu Leu Val Ser	
580	585	590
Asp Gly Gly Pro Asn Leu Tyr	Asn Ile Arg Asn Leu His Ile Pro Glu	

Printed 03-12-2000

SPECIMEN

00120896

595	600	605
Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu		
610	615	620
Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val		
625	630	635 640
Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu		
645	650	655
Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg		
660	665	670
Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met		
675	680	685
Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser		
690	695	700
Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser		
705	710	715 720
Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys		
725	730	735
Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu		
740	745	750
Leu Arg Arg Gln Lys		
755		

<210> 11
 <211> 716
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 11
 Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
 1 5 10 15
 Ala Glu Arg Ala Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr
 20 25 30
 Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
 35 40 45

Printed 08-12-2008

SPECIES

08-12-2008

Ser Asp Phe His Phe Ile Asn Glu Gln Gly Glu Ser Ile Ile Val Glu
50 55 60

Leu Asp Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
65 70 75 80

Gly Arg Asp Arg Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
85 90 95

Thr Thr Gly Ala Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
100 105 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
115 120 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His
130 135 140

Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145 150 155 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
165 170 175

Thr Ile Arg Gln Glu Met Ala Ser Arg Gly Leu Trp Asp Ser Phe Arg
180 185 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Arg Phe Glu Ile Thr
195 200 205

Gly Thr Met Arg Arg Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Ser
210 215 220

Cys Leu Glu Ile Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225 230 235 240

Tyr Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Lys
245 250 255

Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Ile Arg Leu Pro Asp
260 265 270

Gly Pro Pro Cys Ser Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
275 280 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
290 295 300

Printed: 08-12-2004

SPEC

00-120396

Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro			
305	310	315	320
Tyr Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu			
325	330	335	
Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu			
340	345	350	
Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp			
355	360	365	
Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys			
370	375	380	
Arg Asp Ile Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu			
385	390	395	400
Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu			
405	410	415	
Leu Thr Asn Ser Ile Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val			
420	425	430	
Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala			
435	440	445	
Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr			
450	455	460	
Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe			
465	470	475	480
Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg			
485	490	495	
Lys Thr Asn Leu Tyr Gly Phe Ile Val Lys Gly Arg Ser His Leu Arg			
500	505	510	
Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr			
515	520	525	
Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu			
530	535	540	
Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro			
545	550	555	560

Printed on 12-2001

SPEC

001203

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys
565 570 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
580 585 590

Glu Ser Met Ile Glu Ala Gln Ser Ser Val Lys Glu Lys Asp Met Thr
595 600 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser
610 615 620

Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu
625 630 635 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
645 650 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu
660 665 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
675 680 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
690 695 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Arg
705 710 715

<210> 12

<211> 582

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 12

Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp
1 5 10 15

Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
20 25 30

Thr Ile Leu Glu Gln Asn Val Thr Val Thr His Ala Lys Asp Ile Leu
35 40 45

Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
50 55 60

00120898

SPEC

00120898

Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
65 70 75 80

Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu
85 90 95

Lys Glu Asn Pro Arg Asp Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp
100 105 110

Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys
115 120 125

Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
130 135 140

Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn
145 150 155 160

Met Val Trp Leu Thr Lys Lys Glu Ser Asn Tyr Pro Val Ala Lys Gly
165 170 175

Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val
180 185 190

His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val
195 200 205

Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr
210 215 220

Pro Asp Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met
225 230 235 240

Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu
245 250 255

Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys
260 265 270

Arg Gly Asn Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys
275 280 285

Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro
290 295 300

Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val
305 310 315 320

Printed 03/2/2008

SPEC

001268

Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Pro Arg Asn Val Pro Gln
325 330 335

Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
340 345 350

Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn
355 360 365

Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala
370 375 380

Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn
385 390 395 400

Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg
405 410 415

Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp
420 425 430

Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu
435 440 445

Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met
450 455 460

Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe
465 470 475 480

Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr
485 490 495

Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu
500 505 510

Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Tyr Gln Ile Leu Ala
515 520 525

Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala
530 535 540

Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile
545 550 555 560

Cys Ile

03-12-2000

SPEC

00120896

<210> 13
 <211> 506
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 13

Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
 1 5 10 15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
 20 25 30

Ile Asp Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
 35 40 45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
 50 55 60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
 65 70 75 80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
 85 90 95

Tyr Lys Arg Val Asn Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp
 100 105 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp
 115 120 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn
 130 135 140

Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
 145 150 155 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
 165 170 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu
 180 185 190

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
 195 200 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn

Page 103-2-2001

SPECIAL

001238

210	215	220
Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp		
225	230	235 240
Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu		
	245	250 255
Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His		
	260	265 270
Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Thr Ala Val Ala Ser Gly		
	275	280 285
Tyr Asp Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe		
	290	295 300
Lys Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu		
	305	310 315 320
Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala		
	325	330 335
Ala Phe Glu Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Lys Val		
	340	345 350
Ile Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn		
	355	360 365
Glu Asn Met Asp Thr Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg		
	370	375 380
Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg		
	385	390 395 400
Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg		
	405	410 415
Asn Leu Pro Phe Asp Lys Thr Thr Ile Met Ala Ala Phe Thr Gly Asn		
	420	425 430
Ala Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met		
	435	440 445
Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe		
	450	455 460
Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp		

Printed 03-12-2000

SPEC

00120896

465 470 475 480
Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
 485 490 495

Asp Asn Xaa Gly Lys Ile Pro Leu Phe Leu
 500 505

<210> 14
<211> 469
<212> FRT
<213> Influenza virus A/Singapore/1/97/ca

<400> 14
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Ser Leu Thr
1 5 10 15

Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr
 20 25 30

Val Thr Leu His Phe Lys Gln His Glu Cys Asp Ser Pro Ala Ser Asn
 35 40 45

Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
 50 55 60

Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu
65 70 75 80

Val Val Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
 85 90 95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
 100 105 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Gly Lys
 115 120 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Tyr Asn Lys His
130 135 140

Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met
145 150 155 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Val
 165 170 175

Printed: 03-12-2001

SPEC

001208

Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
180 185 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp
195 200 205

Gly Arg Leu Val Asp Ser Ile Gly Ser Trp Ser Gln Asn Ile Leu Arg
210 215 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225 230 235 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
245 250 255

Ile Lys Glu Gly Lys Ile Val Arg Ile Ser Pro Leu Ser Gly Ser Ala
260 265 270

Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asp Val Arg
275 280 285

Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
290 295 300

Ile Asn Met Glu Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly
305 310 315 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Asn Ser Asn
325 330 335

Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
340 345 350

Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly Arg Thr Ile Asn Lys
355 360 365

Asp Ser Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ser
370 375 380

Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn
385 390 395 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
405 410 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln
420 425 430

00120896

SPEC

00120896

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
435 440 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
450 455 460

Asn Phe Met Pro Ile
465

<210> 15

<211> 252

<212> BRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 15

Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
1 5 10 15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
20 25 30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
35 40 45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
50 55 60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65 70 75 80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala
85 90 95

Val Lys Leu Tyr Lys Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
100 105 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
115 120 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
130 135 140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser His His Arg
145 150 155 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
165 170 175

Printed on 12-2001

SPEC

001208

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
180 185 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
195 200 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
210 215 220

Ser Ser Ala Gly Leu Lys Asp Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225 230 235 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
245 250

<210> 16

<211> 97

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 16

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly
1 5 10 15

Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile
20 25 30

Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe
35 40 45

Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser
50 55 60

Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln
65 70 75 80

Gln Ser Ala Val Asp Ala Asp Asp Ser His Phe Val Ser Ile Glu Leu
85 90 95

Glu

<210> 17

<211> 237

<212> PRT

Printed: 03-12-2004

SPC

00120895

<213> Influenza virus A/Singapore/1/57/ca

<400> 17

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp
1 5 10 15

His Val Arg Lys Gln Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe
20 25 30

Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser
35 40 45

Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile
50 55 60

Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr
65 70 75 80

Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu
85 90 95

Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ser
100 105 110

Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile
115 120 125

Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu
130 135 140

Ile Leu Leu Arg Ala Phe Thr Glu Glu Gly Ala Ile Val Gly Glu Ile
145 150 155 160

Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn
165 170 175

Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val
180 185 190

Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Asn Ser Asn Glu
195 200 205

Asn Gly Arg Pro Pro Leu Thr Pro Lys Gln Lys Arg Lys Met Ala Arg
210 215 220

Thr Ile Arg Ser Lys Val Arg Arg Asn Lys Met Ala Asp
225 230 235

<210> 18
 <211> 121
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 18
 Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met
 1 5 10 15
 Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile
 20 25 30
 Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Thr
 35 40 45
 Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys
 50 55 60
 Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile
 65 70 75 80
 Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln
 85 90 95
 Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu
 100 105 110
 Ile Arg Thr Phe Ser Phe Gln Leu Ile
 115 120

<210> 19
 <211> 2396
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

<400> 19
 agcagaagcg gagcgttttc aagatgacat tggctaaaat tgaattgtta aaacaactgt 60
 taaggggacaa tgaagccaaa acagtattga aacaaacaac agtagatcaa tataacataa 120
 taagaaaatt caatacatca agaattgaaa agaacccttc attaaggatg aagtgggcaa 180
 tgtgtttctaa ttttccttgg gctttgacca aggggtgacat ggcaaacaga atcccttgg 240
 aatacaaggg aatacaactt aaacaactat ctgaagacat aggaaccaaa ggccaaatgt 300
 gctcaatagc agcagttacc tgggtggaata catatggacc aataggagat actgaaggtt 360
 tcgaaaaagt ctacgaaagc ttttttctca gaaagatgag acttgacaat gacacttggg 420
 gccgaataac ttttggccca gttgaaaagag taagaaaaag ggtactgcta aaccctorca 480
 ccaagggaat gctccagat gaagcaagta atgtgataat ggaastattg ttccctaagg 540
 aagcaggaat accaagagaa tctacttggg tacataggga actgataaaa gaaaaaagag 600

Printed: 03-12-2007

SPEC

00120896

```

aaaaattgaa aggaacgatg ataactccca ttgtactggc atacatgctt gagagggaa 660
tggttgccag gagaagggtc ctgcccgtag caggagcaac atcagctgag ttcatagaaa 720
tgctaactg cttacaaggt gaasaltgga gacaaatata tcacccggga ggaataaac 780
taactgaatc taggtctcaa tcatgattg tggcttgtag aaagataatc agaagatcaa 840
tagtcgcatc aaaccattg gagctagctg tagaaattgc aaacagact gtaatagata 900
ctgaaccttt aaaatcatgt ctgacagcca tagccggagg tgatgtcgcc tgtgacataa 960
taagggctgc attaggacta aagatcagac aaagacaaag atttggacga ctggaactaa 1020
agagaatata aggaagagga ttcaaaaatg atgaagaaat attaatcggg aacggaaaca 1080
tacagaagat tggaatatgg gacggagaag aggagtcca tgaagatgt ggtgaatga 1140
ggggaatatt aaaaagagc aaatgaqaa tggaaaaact actaataaat tcagctaaaa 1200
aggaagacat gaaagattta ataactctgt gcatggtatt ttctcaagac actaggatgt 1260
tccagaggat gaggggagaa ataaatttct ttaatatagc aggcocactt ttatctcaa 1320
tgtatcaact ccaagatat ttttgaata gaagtaatga tctctttgat caatgggggt 1380
atgagggaatc acccaagca agtgaagctc atgggataaa tgaattaatg aatgcatctg 1440
actacacttt gaaaggggtt gtagtaacaa aaatgtgat tgatgatttt agttctactg 1500
aaacgaaaa agtatctata acaaaaaatc ttagtttaat aaaaaggact ggggaagtca 1560
taatgggagc caatgacgta agtgaattag aatcacaaag tcagctaatg ataactatg 1620
atacacotaa gatgtgggag atgggaacaa ccaagaact ggtgcaaac acctatcaat 1680
gggtgctgaa aaatttgta acactgaag otcagtttct tctaggaaaa gaagaatgt 1740
tccaatggga tgcatttgaa gcatttgaaa gcataatcc ccagaagatg gctggccaat 1800
aoagtggatt tgcagagca gtgctcaaac aaatgagaga ccaagaggtc atgaaaaactg 1860
aocagttoat aaagtgttg cccttttgtt tctcacccgc aaagttaagg agcaatgggg 1920
agccttatca gttcttgagg ctgtattga agggaggagg agaaaatttc atcgaaagta 1980
ggaaagggtc tctctatttc tcttacaatc cacaacaga agtcctaact atatgcggca 2040
gaatgatgtc attaaaaggg aaattgaag atgaagaaag gaatagatca atggggaatg 2100
cagtgttggc gggttttctt gttagtggca agtatgacc agatcctgga gatctcaaaa 2160
ctattgaaga gcttgaaaag ctaaaaccgg gggagaaagc aaacatctta ctttatcaag 2220
gaagcccggt taaagtgtt aaaaggaaaa gatatagtgc tttatccaat gacatttcac 2280
aaggaattaa gagacaaaga atgacagttg agtccatggg gtgggccttg agctaataa 2340
aatttatcaa ttaattcaat gaatgcaat gagtgaaaaa tgctcgtgtt tctcat 2396

```

<210> 20
 <211> 2369
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

```

<400> 20
agcagaagcg gagccttta gatgaatata aatccttatt ttctcttcat agatgtaccc 60
atacaggcag caatttcaac aacattocca tacaccgggt ttccocctta ttccaatgga 120
acgggaacag gccacacaat agacaccgtg atcagaacac atgagtactc gaacaaagga 180
aaacagtatg tttctgacat cacaggatgt acaatgtag atccacaaa tggacoatta 240
cccgaagaca atgagccaag tgccatgca caattagatt gcgttctgga ggctttggat 300
agaatggatg aggaacatcc aggtctgttt caagcagcct cacagaatgc catggaggca 360
ctaattggtc caactgtaga caaattaacc caggggagac agactttcga ttggacagta 420
tgcaagaaatc agcctgtgc aacggcacta aacacaaa aa taacctcctt taggttgaat 480
gatttgaatg gagotgacaa ggggtgattg gtacctttt gccagatat cattgattca 540
ttagacaagc ctgaaatgac tttcttctca gtaagaaata taagaaaaa attocctgct 600
aaaaacaga agggtttctt cataaagaga ataccaatga aagtaaaaga caggatatcc 660

```

Printed 12-2000

SPC-1

001200

```

agagtggaa acatcaaaag agcattgtca ttaaacacaa tgacaaaaga tgcgtgaaag 720
ggcaaaactaa aaagaagagc gattgcaacc gctggaatac aaatcagagg gtttgtatta 780
gtagttgaaa acttggctaa aatatctgt gaaaatctag aacaaagtgg tttgcccgta 840
ggtggaaatg aaaagaaggc caaactgtca aatgcagtgg ccaaaatgct cagtaactgc 900
ccaccaggag ggatcagcat gacagtaaca ggagacaata ctaaatggaa tgaatgctta 960
aatccaagag cctttttggc tatgactgaa agaataacaa gagacagccc aatttggttc 1020
cgggattttt gtagtatagc accggtcttg tcttcoata aatagccag attgggaaaa 1080
ggattttatg caacaagtaa aacaaaaaga ctgaaggctc aaataccttg tectgatctg 1140
tttagcatac cattagaaag atataatgaa gaaacaaggc caaaattaaa aaagctgaaa 1200
ccattcttca atgaagaagg aacggcatct ttgtgcctg gaatgatgat gggaaatgtt 1260
aatatgctat ctacgtgtt gggagtagca gcactaggta tcaaaaacat tggaaacaag 1320
gaatacttat gggatggact gcaatcttct gatgattttg ctctgtttgt taatgcamaa 1380
gatgaagaga catgtatgga aggaataaac gatttttacc gaacatgtaa attattgga 1440
ataaacatga gcaaaaagaa aagttactgt aacgaaactg gaatgtttga atttacaagc 1500
atgttctata gagatggatt tgtatctaac ttgcaatgg aatttcttc atttggagtt 1560
gctggagtaa atgaatcagc agatatggca ataggaatga caataataa gaacaatatg 1620
attaacaatg ggatgggtcc agcaacagca caaacagcca tacaattgtt catagctgat 1680
tataggtaca cctacaaatg ccacagagga gattccaaag tggaaaggaa aagaatgaa 1740
attataaagg agctatggga aaacactaaa ggaagagatg gctgttagt ggcagatggt 1800
gggcccacaa tttaaattt gagaaactta catatcccag aaatagattt gaagtacaat 1860
ctaattggac ctgaatacaa agggcggtta cttcaccttc aaatccctt ttaggacat 1920
ttgtctattg aaggcatcaa agaagcagat ataaccocag oacatggtcc tgtgaagaa 1980
atggattatg atgcagtgtc tggaaactcat agttggagaa ccaaaaggaa cagatctata 2040
ctaatactg atcagaggaa catgatctt gaggaacaat gctacgctaa atgttgcaac 2100
ctttttgag cctgttttaa cagtgcacaa tacaggaac cagtaggcca gcacagcat 2160
cttgaggcta tggcccatag attaagaatg gatgcacgac tggattatga atcaggaaga 2220
atgtcaaagg atgattttga gaaagcaatg gctcaccttg gtgagattgg gtacacataa 2280
gctccgaaga tgtccatggg gttattggtc atcactggat acatgtgata acaaatgat 2340
taaaatgaaa aaaggctcgt gtttctact
2369

```

<210> 21
 <211> 2305
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

```

<400> 21
agcagaagcg gtgcgtttga tttgtcataa tggatacttt tattacaaga aacttccaga 60
ctacaataat acaaaaggcc aaaaacacaa tggcagaatt tagtgaagat cctgaattac 120
aacccgcaat gctattcaac atctgcgtcc atctagaggt ttgctatgta ataagtga 180
tgaattttct tgacgaagaa ggaaaagcat atcagcatt agaaggacaa gggaaagaa 240
aaaatttgag accacaatat gaagttaatt agggaaatgcc aagaaccata gcatggatgg 300
tccaaagatc cttagctcaa gagcatggaa tagagactcc caagtatctg gctgatttgt 360
ttgattataa aaccaagaga ttatagaag ttggaataac aaaaggattg gctgatgatt 420
acttttggaa aaagaaagaa aagctgggaa atagcatgga actgatgata ttcagctaca 480
atcaagacta ttcgttaagt aatgaatcot cattggatga ggaagggaaa gggagagtgc 540
taagcagact cacagaactt caggctgaat taagtctgaa aaacctatgg caagttctca 600
taggagaaga agatgttgaa aagggaattg actttaact tggacaaaca atatctagac 660

```

11110-03-12-2001

SPECTRUM

00120896

taaggatata atctgttcca gctggtttct ccaattttga aggaatgagg agctacatag 720
 acaatataga cccgaaagga gcaatagaga gaaatctagc aaggatgtct cccctagtat 780
 cagtcacacc taaaaagtgt aatgggagg acctagacc aatagggcct cacatttaca 840
 accatgagct accagaagt ccatataatg cctttcttct aatgtctgat gaactggggc 900
 tggccaatat gactgaggga aagtccaaaa aaccgaagac attagccaaa gaatgtctag 960
 aaaaagtact aacactacgg gatcaaatcg acccaatatt aataatgaaa agcgaaaaag 1020
 ctaacgaaaa tttoctatgg aagcttttga gagactgtgt aaatacaata agtaatgagg 1080
 aatgagtaa cgagttagag aaaaaccaatt atgccaagtg ggccacaggg gatggattaa 1140
 cataccagaa aataatgaaa gaagtagcaa tagatgacga aacaatgtgc caagaagagc 1200
 ctaaaatccc taacaaatgt agagtggctg ctgggttcca aacagagatg aatctattga 1260
 gcaactgtgc aagtaaaaaa gctctggacc taccagaaat agggccagac gtatgcacccg 1320
 tggagcatgt agggagtgaa agaaggaaat aactttgtta tgaaatcaac tactgttaag 1380
 cctctacagt tatgatgaag tatgtgtttt ttccacttcc attgttgaat gaaagcaatg 1440
 ccagcatggg aaaaatacaa gtaatacaa taaccaatag agtagtaaat gaaaaaggag 1500
 aaagtttoga catgctctat ggtctggcgg ttaaaggaca atctcatctg aggggagata 1560
 ctgatgtgt aacagtgtca actttcgaat ttagtagtac agaccocaga gtggactcag 1620
 gaaagtggcc aaaaataact gtgttttaga ttggctccct atttgtgagt gggagggaaa 1680
 agtctgtgta cctatattgc cgagtgaatg gcacaaataa gatccaaatg aaatggggaa 1740
 tggaaagctag aagatgtctg cttcaatcaa tgcaacaaat ggaagcaatt gttgaacaag 1800
 aatcatcgat acaaggatag gacatgacca aagcttgttt caaggcagac agagttaata 1860
 gccccaaaac ttccagtatt ggaactcaag aaggaaaact agtaaaagga tcttttgaa 1920
 aagcactaag agtaatattt actaaatgtt tgatgcacta tgtatttggg aatgcccaat 1980
 tggagggggt tagtgccgag tctaggagac ttctactgtt gattcaagca ttaaaggaca 2040
 gaaagggccc ttgggtgttc gacttagagg gaatgtattc tggantagaa gaatgtatta 2100
 gtaaccaacc ttgggtaata cagagtgcac actgggtcaa tgaatggtt ggctttgaaa 2160
 aggaggggag taaagtatta gaatcagtag atgaataat ggatgaataa aaggacatag 2220
 tactcaattt agtactattt tgttcattat gtatotaaac atccaataaa aaggacaaa 2280
 aattaaaaat gcacgtgttt ctact 2305

<210> 22

<211> 1882

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 22

agcagaagca gagcatttcc taatatcoac aaaaatgaagg caataattgt actactcatg 60
 gtatgaacat ccaatgcaga tcaaatctgc actgggataa catcgtaaaa ctacactcat 120
 gtggtcaaaa cagctactca aggggaggtc aatgtgactg gtgcgatacc actgacaaca 180
 acaccaacaa aatctcattt tgcaaatctc aaaggaaaca agaccagagg gaaactatgc 240
 ccaacotgtc tcaactgcac agatctggat gtggccttgg gcagaccaat gtgtgtgggg 300
 atcacacott cggaasaagc ttcaatactc caggaagtcg gaactgttac atccggatgc 360
 tttoctataa tgcacgacag aacaaaaatc agaaagctac ccaatcttct cagaggatag 420
 gaaaaaatca gattataaac ccaaaactgt atcaacacag aaaaaggcac agggagacac 480
 tacagacttg gaacttcagg atcttgcctt aacgotacca gtaaaagcgg atttttcgca 540
 acaatggctt gggctgtccc aagggacaac acaaaaacag caacgaatcc actaacagta 600
 gaagtaccac acatctgtac aaaaagaaga gaccaaatta ctgtttgggg gtccattctt 660
 gataacaaa ccaaatgaa aaacctctat ggagactcaa atcctcaaaa gttcacctca 720
 tctgctaag ggataaccac aattatgtt tctcagattg gcggttccc ggaccaaaca 780

Printed: 03-12-2001

SPEC

001208

gaagacggag gggtaccaca aagcggcaga attgtgtgtg attacatggt gcaaaaacct 840
 gggaaaacag gaacaattgt ctatcaaaaga gggattttgt tgcctcaaaa ggtgtggtgc 900
 gcgag-ggca ggagcaaat aataaaaggg tccttgccct taattggtga agcagattgc 960
 cttcacgasa aatacgggtg attaaacaa agcaagccct actacacagg agaactatga 1020
 aaagccatag gaaattgcc aatatgggtg aaaacacctt tgaagcttgc caatggaacc 1080
 aatatagac ctctgcasa actattaaag gaaaggggtt tcttcgggc tattgctggt 1140
 ttcttagaag gaggatggga aggaatgatt gcaggttggc acggatacac atctcacgga 1200
 gcacatggag tggcagtgge agcagacctt aagagtacgc aagaagccat aaacaagata 1260
 acaaaaatc tcaattcttt gagttagcta gaagtaata accttcaaag actaagtgtg 1320
 gcoatggatg aactccataa cgaataatc gagctggatg agaagtgga tgatctcaga 1380
 gotgacacaa taagctcaca aatagaactt gcagtcctgc tttccaaaga aggaataata 1440
 aacagtgaag atgagcatct attggcactt gagagaaaac taaagaaat gctgggtccc 1500
 tctgctgtag acatagggaa tggatgcttc gaaaccaaac zcaagtga ccagacctgc 1560
 ttagacagga tagctgctgc cactttaat gcagaagaat tttctcttc caottttgat 1620
 tcaatgaaca ttactgctgc atctttaat gatgatggat tggataacca tactatactg 1680
 ctctactact caactgctgc ttctagtgtt gctgtaacat tgatgatagc tatttttatt 1740
 gtttatatga tctccagaga caatgtttct tgctccatct gtctataagg aaatttaagc 1800
 octgtatttt cctttattgt ggtgttgtt tgctgttat cattacaaag aaactgtatt 1860
 gaaaaatgct ctgttacta ct 1882

<210> 23

<211> 1844

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 23

agcagaagca cagcatttcc ttgtgaactt caagtaccag taaaagaact gaaantcaa 60
 atgtcoaaca tggatattga cgttatcaac actgggacaa ttgacaaac accggaagaa 120
 ataaactttt gaaocagtgg gacaaccaga ccaatcatca gaccagcaac ccttgcccca 180
 ccaagcaaca aacgaacctg taacctatcc ccggaagag caaocaaag cagtgaagct 240
 gatgtcggga gyaaaacca aaagaacag acccagacag agataaagaa gagcgtctac 300
 aacatggtag tgaactggg cgaattctac aacagatga tggtaaaagc tggactcaac 360
 gatgacatgg agagaacot aatccaaat gcgcatgctg tggaaagaat tctattggct 420
 gcaactgatg acaagaaaac tgaattccag aagaaaaaga ataccagaga tgtcaaagaa 480
 gggaaagaag aatatagatca caacaaaca ggaggaacct tttaacagat ggttaagat 540
 gataaaacca tctacttcag cctataaga attacctttt taaagaaga ggtgaaaaca 600
 atgtacaaaa coacctggg gatgatggc ttcagtggac taatcacat aatgattggg 660
 cattcacaga tgaatgatgt ctgtttocaa agatcaaaag cactaaaaag agttggaact 720
 gaccttcat taatcagtag ctttgcggga agcaaatcc coagaagato aggtgcaact 780
 ggtgttgcaa tcaaggagg tgaacttta gtggtgaag ccattcgatt tataggaaga 840
 gcaatggcag acagagggct attgagagac atcaaaagca agactgcta tgaagagatt 900
 cttctgaatc taaaaacaa atgtctgog ccoaacaaa aggtctatgt tgatcaagtg 960
 atcggaagta gaaatocagg gattgcagac attgaagatc taacctgct tgctcgtagt 1020
 atggtcgttg itaggccctc tggggcagc aaagtgggtg ttocataag catttacgc 1080
 aaaatacctc aactaggggt caatgttga gagtactota tggttgggta cgaagccatg 1140
 gctotttaca atatggcaac acctgtttcc atattaagaa tgggagatga tgaasaagat 1200
 aagtgcact tattctcat gcttgcctt ggagctgcct atgaagacct gagagttttg 1260
 tctgcattaa caggccacaga attcaagcct agatcagcat taaatgcaa gggtttccat 1320

gttccagcaa aggaacaggt ggaaggaatg ggggagctc tgatgtccat caagctccag 1380
 ttttgggtc caatgaccag atctggggg aacgaagtag gtggagacgg agggctctggc 1440
 caaataagct gcagcccagt gtttgcaagt gaaagaccta ttgotctaag caagcaagct 1500
 gtaagaagaa tgctgtcaat gaatattgag ggacgtgatg cagatgtcaa aggaatcta 1560
 atcaagatga tgaatgactc aatggotaag aaaaccagt gaaatgcttt cattgggaag 1620
 aaatgtctc aaatatcaga caaaaacaaa accaatcccg ttgaaattcc aattaagcag 1680
 accatcccca atttcttctt tgggagggac acagcagagg attatgatga cctcgattat 1740
 taaagcaaca aaatagcaac tatgactgtg attgtttcaa tacgtttgga atgtgggtgt 1800
 ttattcttat taaaataaat ataaaaaat ctgtgtttc tact 1844

<210> 24

<211> 1557

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 24

agcagaagca gaggatcttc tcaasactga ggcaaatagg ccaaaaatga acaatgctac 60
 cttcaactat acaaacgtta accctattcc tcacatcagg gggagtgtta ttatcaatat 120
 atgtgtcagc ttcactgtca tacttattat attcggatat attgctaaaa ttttcaacca 180
 cagaataaac tgcaccaaca atgcoattgg attgtgcaaa cgcattcaat gttcaggctg 240
 tgaaccgttc tgcacaacaa ggggtgacac ttcttctccc agaaccggag tggacatacc 300
 cgcgtttatc ttgccggggo tcaacotttc agaaagcaact cctaattagc cctcatagat 360
 tcggagaaac caaaggaaac tcagctccct tgataataag ggaacctttt attgcttgtg 420
 gaccaaaagga atgcaaacac tttgtcttaa ccoattatgc agoccaaaca gggggatact 480
 acaatggaac aagagaagac agaaucaagc tgaggcatot aatttcagtc aaattgggca 540
 aaatcccaac agtagaaaac tcoattttcc acatggcagc atggagcggg tccgcatgco 600
 atgatggtaa agaattggaca tatacggag ttgatggccc tgacagtaat goattgtcta 660
 aaataaataa tggagaagca tatactgaca cataccattc ctatgcaaac aacatcctaa 720
 gaacacaaga aagtgcctgc aattgcacgt ggggaaattg ttatcttatg ataactgatg 780
 gtcagcttc aggtactagt gaatgcagat ttottaagat tcaagagggc cgaataataa 840
 aagaatatatt tccaacagga agagtagaac atactgaaga atgcacatgc ggatttgcca 900
 gcaataaaac catagaatgt gctgtagag ataacagtta cacagcaaaa agacctttg 960
 tcaaatataa tgtggagact gatacagcag aaataagatt gatgtgcaca gagacttact 1020
 tggacacccc cagaccagat gatggaagca taacagggco ttgtgaatct aatggggata 1080
 aagggagtgagg aggcataaag ggaggatttg tcoatcaag aatggcatcc aagactggaa 1140
 ggtggtactc tcgaacaatg tctaaaacta aaaggatggg gatgggactg tatgtcaagt 1200
 atgatggaga cccatggact gacagtgatg cccttgctct tagtggagta atggtttcaa 1260
 tggagaagacc tggttggtac tcccttggtt tcgaataaaa agataagaaa tgtgatgtcc 1320
 cctgtattgg gatagagatg gtacatgatg gtggaaagga gacttggcac tcagcagcaa 1380
 cagccattta ctgtttaatg ggctcaggac aactgctatg ggacactgtc acaggtgtta 1440
 atatggctct gtaattggagg aatggttgag tctgttctaa accctttgtt corattttgt 1500
 ttgaacaatt gtccttactg aacttaattg tttctgaaaa atgctcttgt tactact 1557

<210> 25

<211> 1190

<212> DNA

<213> Influenza B/Vienna/1/99/ca

Revised: 03-12-2001

SPEC

10012005

<400> 25

agcagaagca cgcactttct taagatgtcg ctgtttggag acacaattgc ctacctgctt 60
tcattgacag aagatggaga aggcacaagca gaactagcag aaaaattaca ctgttggttc 120
ggtgggaag aatttgacct agactctgcc ttggaatgga taaaaaaca aagatgctta 180
actgatatac aaaaagcact aattggtgcc tctatctgct ttttaaaac caaagaccag 240
gaaagaaaaa gaagattcxt cacagagcgo ctatcaggaa tgggaacaac agcaacaaaa 300
aagaaaaggoc tgattctagc tgagagaaaa atgagaagat gtgtgagctt tcatgaagca 360
tttgaaatag cagaaggcca tgaaagctca gcgctactat attgtctcat ggtcatgtac 420
ctgaatcctg gaaattatc aatgcaagta aaactaggaa cgtctctgct tttgtgcgag 480
aaacaagcat cacttcaca cagggtcat agcagagcag cgagatcttc agtgcctcga 540
gtgagacgag aatgcagat ggtctcagct atgaacacag caaaaaaant gaatggatg 600
ggaaaaggag aagacgtcca aaaaactggc gaagagctgc aaagcaacat tggagtactg 660
agatctcttg gggcaagtca aaagaatggg gaagggaattg caaaggatgt aatggagtg 720
ctaaagcaga gctctatggg aaattcagct ctgtgaga aatatctata atgctcgaac 780
catttcagat totttcaatt tgtttcttta tcttatcago tctccatttc gtggcttggg 840
caatagggca tttgaatcaa ataaaaagag gagtaaacat gaaantacga ataaaaagtc 900
caaacaaaga gacaataaac agagaggtat caattttgag acacagttac caaaaagaaa 960
tccaggccaa agaaacantg aaggagtag tctctgacaa catggaggta ttgggtgacc 1020
acatagtaat tgaggggctt totgcgaag agataatasa aatgggtgaa acagttttgg 1080
agatagaaga attgcattaa attcaatttc tactgtattt ctactatgc atttaagcaa 1140
attgtaatca atgtcagcaa ataaactgga aaaagtgcgt tgtttctact 1190

<210> 26

<211> 1097

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 26

agcagaagca gagcatttgt ttagtcaactg gcasacagga aaaatggcga acaacataac 60
cacaacacaa attgaggtgg gtccgggagc aaccaatgcc accataaat ttgaaacagg 120
aattctggag tgctatgaaa ggctttcatg gcaaaagacc cttgactacc ctggtcaaga 180
ccgcctaacc agactazaga gaaaattaga gtcaagaata aagactcaca acaaaagtga 240
gcctgaaagt aaaaggatgt ctcttgaaga gaggaagca attggagtaa aaatgatgaa 300
agtaactcta tttatgaatc catctgctgg aattgaaggg tttgagccat actatatgaa 360
aagttoctca aatagcact gtccgaaata caattggacc gattaccctt caacaccagg 420
gaggtgcctt gatgacatag aagaagaacc agaggatgtt gatggccaa ctgaaatagt 480
attaagggac atgaacaaca aagatgcaag gcaaaagata aaagagggaag taaacactca 540
gaaagaaggg aagtccgtt tgacaataaa aagggatata cgtaatgtat tgtccttgag 600
agtgttggtt aacggaacat tcttcaaaa cccaatgga tacaagtcct tatcaactct 660
gcatagattg aatgcatatg accagagtgg aaggcttgtt gctaaacttg ttgctactga 720
tgatottaca gtggaggatg aagaagatgg ccacgggato otaaaactca tcttcgagcg 780
tottaatgaa ggacattcaa agccaattog agcagctgaa actgctgttg gagtcttate 840
ccaatttggc caagagcaco gattatcacc agaagaggga gacaattaaa ctggtcacag 900
aagaacttta tottttaagt aaaaagattg atgataacat attgttccac aaacacgtaa 960
tagctaacag ctccataata gctgacatgg ttgtatcatt atcattatta gaaaattgt 1020
atgaaatgae ggatgtgggtt gaagtgcaca gcaggcagtg cttgtgaatt taaaataaaa 1080
atcctcttgt tactact 1097

Printed 03/12/2001

SPEC

00120898

<210> 27
<211> 770
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 27
Met Thr Leu Ala Lys Ile Glu Leu Leu Lys Gln Leu Leu Arg Asp Asn
1 5 10 15
Glu Ala Lys Thr Val Leu Lys Gln Thr Thr Val Asp Gln Tyr Asn Ile
20 25 30
Ile Arg Lys Phe Asn Thr Ser Arg Ile Glu Lys Asn Pro Ser Leu Arg
35 40 45
Met Lys Trp Ala Met Cys Ser Asn Phe Pro Leu Ala Leu Thr Lys Gly
50 55 60
Asp Met Ala Asn Arg Ile Pro Leu Glu Tyr Lys Gly Ile Gln Leu Lys
65 70 75 80
Thr Asn Ala Glu Asp Ile Gly Thr Lys Gly Gln Met Cys Ser Ile Ala
85 90 95
Ala Val Thr Trp Trp Asn Thr Tyr Gly Pro Ile Gly Asp Thr Glu Gly
100 105 110
Phe Glu Lys Val Tyr Glu Ser Phe Phe Leu Arg Lys Met Arg Leu Asp
115 120 125
Asn Ala Thr Trp Gly Arg Ile Thr Phe Gly Pro Val Glu Arg Val Arg
130 135 140
Lys Arg Val Leu Leu Asn Pro Leu Thr Lys Glu Met Pro Pro Asp Glu
145 150 155 160
Ala Ser Asn Val Ile Met Glu Ile Leu Phe Pro Lys Glu Ala Gly Ile
165 170 175
Pro Arg Glu Ser Thr Trp Ile His Arg Glu Leu Ile Lys Glu Lys Arg
180 185 190
Glu Lys Leu Lys Gly Thr Met Ile Thr Pro Ile Val Leu Ala Tyr Met
195 200 205
Leu Glu Arg Glu Leu Val Ala Arg Arg Arg Phe Leu Pro Val Ala Gly
210 215 220

Printed: 03-12-2001

SPEC

001208

Ala Thr Ser Ala Glu Phe Ile Glu Met Leu His Cys Leu Gln Gly Glu
225 230 235 240

Asn Trp Arg Gln Ile Tyr His Pro Gly Gly Asn Lys Leu Thr Glu Ser
245 250 255

Arg Ser Gln Ser Met Ile Val Ala Cys Arg Lys Ile Ile Arg Arg Ser
260 265 270

Ile Val Ala Ser Asn Pro Leu Glu Leu Ala Val Glu Ile Ala Asn Lys
275 280 285

Thr Val Ile Asp Thr Glu Pro Leu Lys Ser Cys Leu Thr Ala Ile Asp
290 295 300

Gly Gly Asp Val Ala Cys Asp Ile Ile Arg Ala Ala Leu Gly Leu Lys
305 310 315 320

Ile Arg Gln Arg Gln Arg Phe Gly Arg Leu Glu Leu Lys Arg Ile Ser
325 330 335

Gly Arg Gly Phe Lys Asn Asp Glu Glu Ile Leu Ile Gly Asn Gly Thr
340 345 350

Ile Gln Lys Ile Gly Ile Trp Asp Gly Glu Glu Glu Phe His Val Arg
355 360 365

Cys Gly Glu Cys Arg Gly Ile Leu Lys Lys Ser Lys Met Arg Met Glu
370 375 380

Lys Leu Leu Ile Asn Ser Ala Lys Lys Glu Asp Met Lys Asp Leu Ile
385 390 395 400

Ile Leu Cys Met Val Phe Ser Gln Asp Thr Arg Met Phe Gln Gly Val
405 410 415

Arg Gly Glu Ile Asn Phe Leu Asn Arg Ala Gly Gln Leu Leu Ser Pro
420 425 430

Met Tyr Gln Leu Gln Arg Tyr Phe Leu Asn Arg Ser Asn Asp Leu Phe
435 440 445

Asp Gln Trp Gly Tyr Glu Glu Ser Pro Lys Ala Ser Glu Leu His Gly
450 455 460

Ile Asn Glu Leu Met Asn Ala Ser Asp Tyr Thr Leu Lys Gly Val Val
465 470 475 480

Printed: 03-12-2001

SPEC

00120896

Val Thr Lys Asn	Val Ile Asp Asp Phe Ser Ser Thr Glu Thr Glu Lys
485	490 495
Val Ser Ile Thr Lys Asn Leu Ser Leu Ile Lys Arg Thr Gly Glu Val	
500	505 510
Ile Met Gly Ala Asn Asp Val Ser Glu Leu Glu Ser Gln Ala Gln Leu	
515	520 525
Met Ile Thr Tyr Asp Thr Pro Lys Met Trp Glu Met Gly Thr Thr Lys	
530	535 540
Glu Leu Val Gln Asn Thr Tyr Gln Trp Val Leu Lys Asn Leu Val Thr	
545	550 555 560
Leu Lys Ala Gln Phe Leu Leu Gly Lys Glu Asp Met Phe Gln Trp Asp	
565	570 575
Ala Phe Glu Ala Phe Glu Ser Ile Ile Pro Gln Lys Met Ala Gly Gln	
580	585 590
Tyr Ser Gly Phe Ala Arg Ala Val Leu Lys Gln Met Arg Asp Gln Glu	
595	600 605
Val Met Lys Thr Asp Gln Phe Ile Lys Leu Leu Pro Phe Cys Phe Ser	
610	615 620
Pro Pro Lys Leu Arg Ser Asn Gly Glu Pro Tyr Gln Phe Leu Arg Leu	
625	630 635 640
Val Leu Lys Gly Gly Gly Glu Asn Phe Ile Glu Val Arg Lys Gly Ser	
645	650 655
Pro Leu Phe Ser Tyr Asn Pro Gln Thr Glu Val Leu Thr Ile Cys Gly	
660	665 670
Arg Met Met Ser Leu Lys Gly Lys Ile Glu Asp Glu Glu Arg Asn Arg	
675	680 685
Ser Met Gly Asn Ala Val Leu Ala Gly Phe Leu Val Ser Gly Lys Tyr	
690	695 700
Asp Pro Asp Leu Gly Asp Phe Lys Thr Ile Glu Glu Leu Glu Lys Leu	
705	710 715 720
Lys Pro Gly Glu Lys Ala Asn Ile Leu Leu Tyr Gln Gly Lys Pro Val	
725	730 735

Printed: 03-12-2001

SECRET

00-1208

Lys Val Val Lys Arg Lys Arg Tyr Ser Ala Leu Ser Asn Asp Ile Ser
740 745 750

Gln Gly Ile Lys Arg Gln Arg Met Thr Val Glu Ser Met Gly Trp Ala
755 760 765

Leu Ser
770

<210> 28

<211> 752

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 28

Met Asn Ile Asn Pro Tyr Phe Leu Phe Ile Asp Val Pro Ile Gln Ala
1 5 10 15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Val Pro Pro Tyr Ser His
20 25 30

Gly Thr Gly Thr Gly His Thr Ile Asp Thr Val Ile Arg Thr His Glu
35 40 45

Tyr Ser Asn Lys Gly Lys Gln Tyr Val Ser Asp Ile Thr Gly Cys Thr
50 55 60

Met Val Asp Pro Thr Asn Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65 70 75 80

Ala Tyr Ala Gln Leu Asp Cys Val Leu Glu Ala Leu Asp Arg Met Asp
85 90 95

Glu Glu His Pro Gly Leu Phe Gln Ala Ala Ser Gln Asn Ala Met Glu
100 105 110

Ala Leu Met Val Thr Thr Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
115 120 125

Phe Asp Trp Thr Val Cys Arg Asn Gln Pro Ala Ala Thr Ala Leu Asn
130 135 140

Thr Thr Ile Thr Ser Phe Arg Leu Asn Asp Leu Asn Gly Ala Asp Lys
145 150 155 160

Gly Gly Leu Val Pro Phe Cys Gln Asp Ile Ile Asp Ser Leu Asp Lys
165 170 175

Printed 03-12-2000

SECRET

00120898

Pro Glu Met Thr Phe Phe Ser Val Lys Asn Ile Lys Lys Lys Phe Pro
180 185 190

Ala Lys Asn Arg Lys Gly Phe Leu Ile Lys Arg Ile Pro Met Lys Val
195 200 205

Lys Asp Arg Ile Ser Arg Val Glu Tyr Ile Lys Arg Ala Leu Ser Leu
210 215 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225 230 235 240

Ile Ala Thr Ala Gly Ile Gln Ile Arg Gly Phe Val Leu Val Val Glu
245 250 255

Asn Leu Ala Lys Asn Ile Cys Glu Asn Leu Glu Gln Ser Gly Leu Pro
260 265 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ser Asn Ala Val Ala Lys
275 280 285

Met Leu Ser Asn Cys Pro Pro Gly Gly Ile Ser Met Thr Val Thr Gly
290 295 300

Asp Asn Thr Lys Trp Asn Glu Cys Leu Asn Pro Arg Val Phe Leu Ala
305 310 315 320

Met Thr Glu Arg Ile Thr Arg Asp Ser Pro Ile Trp Phe Arg Asp Phe
325 330 335

Cys Ser Ile Ala Pro Val Leu Phe Ser Asn Lys Ile Ala Arg Leu Gly
340 345 350

Lys Gly Phe Met Ile Thr Ser Lys Thr Lys Arg Leu Lys Ala Gln Ile
355 360 365

Pro Cys Pro Asp Leu Phe Ser Ile Pro Leu Glu Arg Tyr Asn Glu Glu
370 375 380

Thr Arg Ala Lys Leu Lys Lys Leu Lys Pro Phe Phe Asn Glu Glu Gly
385 390 395 400

Thr Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu
405 410 415

Ser Thr Val Leu Gly Val Ala Ala Leu Gly Ile Lys Asn Ile Gly Asn
420 425 430

Printed: 03-12-2001

SPR

001288

Lys Glu Tyr Leu Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala Leu
435 440 445

Phe Val Asn Ala Lys Asp Glu Glu Thr Cys Met Glu Gly Ile Asn Asp
450 455 460

Phe Tyr Arg Thr Cys Lys Leu Leu Gly Ile Asn Met Ser Lys Lys Lys
465 470 475 480

Ser Tyr Cys Asn Glu Thr Gly Met Phe Glu Phe Thr Ser Met Phe Tyr
485 490 495

Arg Asp Gly Phe Val Ser Asn Phe Ala Met Glu Ile Pro Ser Phe Gly
500 505 510

Val Ala Gly Val Asn Glu Ser Ala Asp Met Ala Ile Gly Met Thr Ile
515 520 525

Ile Lys Asn Asn Met Ile Asn Asn Gly Met Gly Pro Ala Thr Ala Gln
530 535 540

Thr Ala Ile Gln Leu Phe Ile Ala Asp Tyr Arg Tyr Thr Tyr Lys Cys
545 550 555 560

His Arg Gly Asp Ser Lys Val Glu Gly Lys Arg Met Lys Ile Ile Lys
565 570 575

Glu Leu Trp Glu Asn Thr Lys Gly Arg Asp Gly Leu Leu Val Ala Asp
580 585 590

Gly Gly Pro Asn Ile Tyr Asn Leu Arg Asn Leu His Ile Pro Glu Ile
595 600 605

Val Leu Lys Tyr Asn Leu Met Asp Pro Glu Tyr Lys Gly Arg Leu Leu
610 615 620

His Pro Gln Asn Pro Phe Val Gly His Leu Ser Ile Glu Gly Ile Lys
625 630 635 640

Glu Ala Asp Ile Thr Pro Ala His Gly Pro Val Lys Lys Met Asp Tyr
645 650 655

Asp Ala Val Ser Gly Thr His Ser Trp Arg Thr Lys Arg Asn Arg Ser
660 665 670

Ile Leu Asn Thr Asp Gln Arg Asn Met Ile Leu Glu Glu Gln Cys Tyr
675 680 685

Printed: 03-12-2001

SPEC

00120898

Ala Lys Cys Cys Asn Leu Phe Glu Ala Cys Phe Asn Ser Ala Ser Tyr
690 695 700

Arg Lys Pro Val Gly Gln His Ser Met Leu Glu Ala Met Ala His Arg
705 710 715 720

Leu Arg Met Asp Ala Arg Leu Asp Tyr Glu Ser Gly Arg Met Ser Lys
725 730 735

Asp Asp Phe Glu Lys Ala Met Ala His Leu Gly Glu Ile Gly Tyr Thr
740 745 750

<210> 29

<211> 726

<212> PRT

<213> Influenza B/Vienna/1/99/oa

<400> 29

Met Asp Thr Phe Ile Thr Arg Asn Phe Gln Thr Thr Ile Ile Gln Lys
1 5 10 15

Ala Lys Asn Thr Met Ala Glu Phe Ser Glu Asp Pro Glu Leu Gln Pro
20 25 30

Ala Met Leu Phe Asn Ile Cys Val His Leu Glu Val Cys Tyr Val Ile
35 40 45

Ser Asp Met Asn Phe Leu Asp Glu Glu Gly Lys Ala Tyr Thr Ala Leu
50 55 60

Glu Gly Gln Gly Lys Glu Gln Asn Leu Arg Pro Gln Tyr Glu Val Ile
65 70 75 80

Glu Gly Met Pro Arg Thr Ile Ala Trp Met Val Gln Arg Ser Leu Ala
85 90 95

Gln Glu His Gly Ile Glu Thr Pro Lys Tyr Leu Ala Asp Leu Phe Asp
100 105 110

Tyr Lys Thr Lys Arg Phe Ile Glu Val Gly Ile Thr Lys Gly Leu Ala
115 120 125

Asp Asp Tyr Phe Trp Lys Lys Lys Glu Lys Leu Gly Asn Ser Met Glu

Printed: 03-12-2001

SECRET

10-2089

130	135	140
Leu Met Ile Phe Ser Tyr Asn Gln Asp Tyr Ser Leu Ser Asn Glu Ser		
145	150	155 160
Ser Leu Asp Glu Glu Gly Lys Gly Arg Val Leu Ser Arg Leu Thr Glu		
	165	170 175
Leu Gln Ala Glu Leu Ser Leu Lys Asn Leu Trp Gln Val Leu Ile Gly		
	180	185 190
Glu Glu Asp Val Glu Lys Gly Ile Asp Phe Lys Leu Gly Gln Thr Ile		
	195	200 205
Ser Arg Leu Arg Asp Ile Ser Val Pro Ala Gly Phe Ser Asn Phe Glu		
	210	215 220
Gly Met Arg Ser Tyr Ile Asp Asn Ile Asp Pro Lys Gly Ala Ile Glu		
	225	230 235 240
Arg Asn Leu Ala Arg Met Ser Pro Leu Val Ser Val Thr Pro Lys Lys		
	245	250 255
Leu Lys Trp Glu Asp Leu Arg Pro Ile Gly Pro His Ile Tyr Asn His		
	260	265 270
Glu Leu Pro Glu Val Pro Tyr Asn Ala Phe Leu Leu Met Ser Asp Glu		
	275	280 285
Leu Gly Leu Ala Asn Met Thr Glu Gly Lys Ser Lys Lys Pro Lys Thr		
	290	295 300
Leu Ala Lys Glu Cys Leu Glu Lys Tyr Ser Thr Leu Arg Asp Gln Thr		
	305	310 315 320
Asp Pro Ile Leu Ile Met Lys Ser Glu Lys Ala Asn Glu Asn Phe Leu		
	325	330 335
Trp Lys Leu Trp Arg Asp Cys Val Asn Thr Ile Ser Asn Glu Glu Met		
	340	345 350
Ser Asn Glu Leu Gln Lys Thr Asn Tyr Ala Lys Trp Ala Thr Gly Asp		
	355	360 365
Gly Leu Thr Tyr Gln Lys Ile Met Lys Glu Val Ala Ile Asp Asp Glu		
	370	375 380
Thr Met Cys Gln Glu Glu Pro Lys Ile Pro Asn Lys Cys Arg Val Ala		

Printed 03-12-2000

SPEC 2

00126896

385	390	395	400
Ala Trp Val Gln Thr Glu Met Asn Leu Leu Ser Thr Leu Thr Ser Lys	405	410	415
Lys Ala Leu Asp Leu Pro Glu Ile Gly Pro Asp Val Ala Pro Val Glu	420	425	430
His Val Gly Ser Glu Arg Arg Lys Tyr Phe Val Asn Glu Ile Asn Tyr	435	440	445
Cys Lys Ala Ser Thr Val Met Met Lys Tyr Val Leu Phe His Thr Ser	450	455	460
Leu Leu Asn Glu Ser Asn Ala Ser Met Gly Lys Tyr Lys Val Ile Pro	465	470	475
Ile Thr Asn Arg Val Val Asn Glu Lys Gly Glu Ser Phe Asp Met Leu	485	490	495
Tyr Gly Leu Ala Val Lys Gly Gln Ser His Leu Arg Gly Asp Thr Asp	500	505	510
Val Val Thr Val Val Thr Phe Glu Phe Ser Ser Thr Asp Pro Arg Val	515	520	525
Asp Ser Gly Lys Trp Pro Lys Tyr Thr Val Phe Arg Ile Gly Ser Leu	530	535	540
Phe Val Ser Gly Arg Glu Lys Ser Val Tyr Leu Tyr Cys Arg Val Asn	545	550	555
Gly Thr Asn Lys Ile Gln Met Lys Trp Gly Met Glu Ala Arg Arg Cys	565	570	575
Leu Leu Gln Ser Met Gln Gln Met Glu Ala Ile Val Glu Gln Glu Ser	580	585	590
Ser Ile Gln Gly Tyr Asp Met Thr Lys Ala Cys Phe Lys Gly Asp Arg	595	600	605
Val Asn Ser Pro Lys Thr Phe Ser Ile Gly Thr Gln Glu Gly Lys Leu	610	615	620
Val Lys Gly Ser Phe Gly Lys Ala Leu Arg Val Ile Phe Thr Lys Cys	625	630	635
Leu Met His Tyr Val Phe Gly Asn Ala Gln Leu Glu Gly Phe Ser Ala			

Printed: 03-12-2001

SREC

0012089

645 650 655
Glu Ser Arg Arg Leu Leu Leu Leu Ile Gln Ala Leu Lys Asp Arg Lys
660 665 670
Gly Pro Trp Val Phe Asp Leu Glu Gly Met Tyr Ser Gly Ile Glu Glu
675 680 685
Cys Ile Ser Asn Asn Pro Trp Val Ile Gln Ser Ala Tyr Trp Phe Asn
690 695 700
Glu Trp Leu Gly Phe Glu Lys Glu Gly Ser Lys Val Leu Glu Ser Val
705 710 715 720
Asp Glu Ile Met Asp Glu
725

<210> 30
<211> 384
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 30
Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1 5 10 15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
20 25 30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Ala Ile Pro Leu Thr
35 40 45
Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr
50 55 60
Arg Gly Lys Leu Cys Pro Thr Cys Leu Asn Cys Thr Asp Leu Asp Val
65 70 75 80
Ala Leu Gly Arg Pro Met Cys Val Gly Ile Thr Pro Ser Ala Lys Ala
85 90 95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
100 105 110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
115 120 125

Printed: 03-12-2000

SECRET

00-20496

Tyr Glu Lys Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Thr Glu Lys
130 135 140

Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn
145 150 155 160

Ala Thr Ser Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175

Arg Asp Asn Asn Lys Thr Ala Thr Asn Pro Leu Thr Val Glu Val Pro
180 185 190

His Ile Cys Thr Lys Glu Glu Asp Gln Ile Thr Val Trp Gly Phe His
195 200 205

Ser Asp Asn Lys Thr Gln Met Lys Asn Leu Tyr Gly Asp Ser Asn Pro
210 215 220

Gln Lys Phe Thr Ser Ser Ala Asn Gly Ile Thr Thr His Tyr Val Ser
225 230 235 240

Gln Ile Gly Gly Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
245 250 255

Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Pro Gly Lys Thr
260 265 270

Gly Thr Ile Val Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val Trp
275 280 285

Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile
290 295 300

Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser
305 310 315 320

Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro
325 330 335

Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg
340 345 350

Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala
355 360 365

Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly
370 375 380

Printed: 03-12-2001

SPECIAL

001203

Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys
385 390 395 400

Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu
405 410 415

Ser Glu Leu Glu Val Asn Asn Leu Gln Arg Leu Ser Gly Ala Met Asp
420 425 430

Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu
435 440 445

Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser
450 455 460

Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu
465 470 475 480

Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn
485 490 495

Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg
500 505 510

Ile Ala Ala Gly Thr Phe Asn Ala Glu Glu Phe Ser Leu Pro Thr Phe
515 520 525

Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp
530 535 540

Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala
545 550 555 560

Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Ile Ser Arg Asp
565 570 575

Asn Val Ser Cys Ser Ile Cys Leu
580

<210> 31

<211> 560

<212> PRT

<213> Influenza B/Vienna/1/99/os

<400> 31

Met Ser Asn Met Asp Ile Asp Gly Ile Asn Thr Gly Thr Ile Asp Lys

1

5

10

15

25-09-2001

Printed 03-12-2001

SPECIES

00-120896

Thr Pro Glu Glu Ile Thr Phe Gly Thr Ser Gly Thr Thr Arg Pro Ile
20 25 30

Ile Arg Pro Ala Thr Leu Ala Pro Pro Ser Asn Lys Arg Thr Arg Asn
35 40 45

Pro Ser Pro Glu Arg Ala Thr Thr Ser Ser Glu Ala Asp Val Gly Arg
50 55 60

Lys Thr Gln Lys Lys Gln Thr Pro Thr Glu Ile Lys Lys Ser Val Tyr
65 70 75 80

Asn Met Val Val Lys Leu Gly Glu Phe Tyr Asn Gln Met Met Val Lys
85 90 95

Ala Gly Leu Asn Asp Asp Met Glu Arg Asn Leu Ile Gln Asn Ala His
100 105 110

Ala Val Glu Arg Ile Leu Leu Ala Ala Thr Asp Asp Lys Lys Thr Glu
115 120 125

Phe Gln Lys Lys Lys Asn Thr Arg Asp Val Lys Glu Gly Lys Glu Glu
130 135 140

Ile Asp His Asn Lys Thr Gly Gly Thr Phe Tyr Lys Met Val Arg Asp
145 150 155 160

Asp Lys Thr Ile Tyr Phe Ser Pro Ile Arg Ile Thr Phe Leu Lys Glu
165 170 175

Glu Val Lys Thr Met Tyr Lys Thr Thr Met Gly Ser Asp Gly Phe Ser
180 185 190

Gly Leu Asn His Ile Met Ile Gly His Ser Gln Met Asn Asp Val Cys
195 200 205

Phe Gln Arg Ser Lys Ala Leu Lys Arg Val Gly Leu Asp Pro Ser Leu
210 215 220

Ile Ser Thr Phe Ala Gly Ser Thr Ile Pro Arg Arg Ser Gly Ala Thr
225 230 235 240

Gly Val Ala Ile Lys Gly Gly Gly Thr Leu Val Ala Glu Ala Ile Arg
245 250 255

Phe Ile Gly Arg Ala Met Ala Asp Arg Gly Leu Leu Arg Asp Ile Lys
260 265 270

Printed 03-12-2001

SPEC

0012089

Ala Lys Thr Ala Tyr Glu Lys Ile Leu Leu Asn Leu Lys Asn Lys Cys
275 280 285

Ser Ala Pro Gln Gln Lys Ala Leu Val Asp Gln Val Ile Gly Ser Arg
290 295 300

Asn Pro Gly Ile Ala Asp Ile Glu Asp Leu Thr Leu Leu Ala Arg Ser
305 310 315 320

Met Val Val Val Arg Pro Ser Val Ala Ser Lys Val Val Leu Pro Ile
325 330 335

Ser Ile Tyr Ala Lys Ile Pro Gln Leu Gly Phe Asn Val Glu Glu Tyr
340 345 350

Ser Met Val Gly Tyr Glu Ala Met Ala Leu Tyr Asn Met Ala Thr Pro
355 360 365

Val Ser Ile Leu Arg Met Gly Asp Asp Ala Lys Asp Lys Ser Gln Leu
370 375 380

Phe Phe Met Ser Cys Phe Gly Ala Ala Tyr Glu Asp Leu Arg Val Leu
385 390 395 400

Ser Ala Leu Thr Gly Thr Glu Phe Lys Pro Arg Ser Ala Leu Lys Cys
405 410 415

Lys Gly Phe His Val Pro Ala Lys Glu Gln Val Glu Gly Met Gly Ala
420 425 430

Ala Leu Met Ser Ile Lys Leu Gln Phe Trp Ala Pro Met Thr Arg Ser
435 440 445

Gly Gly Asn Glu Val Gly Gly Asp Gly Gly Ser Gly Gln Ile Ser Cys
450 455 460

Ser Pro Val Phe Ala Val Glu Arg Pro Ile Ala Leu Ser Lys Gln Ala
465 470 475 480

Val Arg Arg Met Leu Ser Met Asn Ile Glu Gly Arg Asp Ala Asp Val
485 490 495

Lys Gly Asn Leu Leu Lys Met Met Asn Asp Ser Met Ala Lys Lys Thr
500 505 510

Ser Gly Asn Ala Phe Ile Gly Lys Lys Met Phe Gln Ile Ser Asp Lys
515 520 525

25-09-2000

Printed: 03/12/2000

SPEC

00120396

Asn Lys Thr Asn Pro Val Glu Ile Pro Ile Lys Gln Thr Ile Pro Asn
530 535 540

Phe Phe Phe Gly Arg Asp Thr Ala Glu Asp Tyr Asp Asp Leu Asp Tyr
545 550 555 560

<210> 32
<211> 100
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 32
Met Asn Asn Ala Thr Phe Asn Tyr Thr Asn Val Asn Pro Ile Pro His
1 5 10 15

Ile Arg Gly Ser Val Ile Ile Thr Ile Cys Val Ser Phe Thr Val Ile
20 25 30

Leu Ile Ile Phe Gly Tyr Ile Ala Lys Ile Phe Thr Asn Arg Asn Asn
35 40 45

Cys Thr Asn Asn Ala Ile Gly Leu Cys Lys Arg Ile Lys Cys Ser Gly
50 55 60

Cys Glu Pro Phe Cys Asn Lys Arg Gly Asp Thr Ser Ser Pro Arg Thr
65 70 75 80

Gly Val Asp Ile Pro Ala Phe Ile Leu Pro Gly Leu Asn Leu Ser Glu
85 90 95

Ser Thr Pro Asn
100

<210> 33
<211> 466
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 33
Met Leu Pro Ser Thr Ile Gln Thr Leu Thr Leu Phe Leu Thr Ser Gly
1 5 10 15

Printed: 03-12-2003

SPECTRA

0012039

Gly Val Leu Leu Ser Leu Tyr Val Ser Ala Ser Leu Ser Tyr Leu Leu
20 25 30

Tyr Ser Asp Ile Leu Leu Lys Phe Ser Pro Thr Glu Ile Thr Ala Pro
35 40 45

Thr Met Pro Leu Asp Cys Ala Asn Ala Ser Asn Val Gln Ala Val Asn
50 55 60

Arg Ser Ala Thr Lys Gly Val Thr Leu Leu Leu Pro Glu Pro Glu Trp
65 70 75 80

Thr Tyr Pro Arg Leu Ser Cys Pro Gly Ser Thr Phe Gln Lys Ala Leu
85 90 95

Leu Ile Ser Pro His Arg Phe Gly Glu Thr Lys Gly Asn Ser Ala Pro
100 105 110

Leu Ile Ile Arg Glu Pro Phe Ile Ala Cys Gly Pro Lys Glu Cys Lys
115 120 125

His Phe Ala Leu Thr His Tyr Ala Ala Gln Pro Gly Gly Tyr Tyr Asn
130 135 140

Gly Thr Arg Glu Asp Arg Asn Lys Leu Arg His Leu Ile Ser Val Lys
145 150 155 160

Leu Gly Lys Ile Pro Thr Val Glu Asn Ser Ile Phe His Met Ala Ala
165 170 175

Trp Ser Gly Ser Ala Cys His Asp Gly Lys Glu Trp Thr Tyr Ile Gly
180 185 190

Val Asp Gly Pro Asp Ser Asn Ala Leu Leu Lys Ile Lys Tyr Gly Glu
195 200 205

Ala Tyr Thr Asp Thr Tyr His Ser Tyr Ala Asn Asn Ile Leu Arg Thr
210 215 220

Gln Glu Ser Ala Cys Asn Cys Ile Gly Gly Asn Cys Tyr Leu Met Ile
225 230 235 240

Thr Asp Gly Ser Ala Ser Gly Ile Ser Glu Cys Arg Phe Leu Lys Ile
245 250 255

Gln Glu Gly Arg Ile Ile Lys Glu Ile Phe Pro Thr Gly Arg Val Glu
260 265 270

Printed: 03-12-2004

SPEC

00120896

His Thr Glu Glu Cys Thr Cys Gly Phe Ala Ser Asn Lys Thr Ile Glu
275 280 285

Cys Ala Cys Arg Asp Asn Ser Tyr Thr Ala Lys Arg Pro Phe Val Lys
290 295 300

Leu Asn Val Glu Thr Asp Thr Ala Glu Ile Arg Leu Met Cys Thr Glu
305 310 315 320

Thr Tyr Leu Asp Thr Pro Arg Pro Asp Asp Gly Ser Ile Thr Gly Pro
325 330 335

Cys Glu Ser Asn Gly Asp Lys Gly Ser Gly Gly Ile Lys Gly Gly Phe
340 345 350

Val His Gln Arg Met Ala Ser Lys Thr Gly Arg Trp Tyr Ser Arg Thr
355 360 365

Met Ser Lys Thr Lys Arg Met Gly Met Gly Leu Tyr Val Lys Tyr Asp
370 375 380

Gly Asp Pro Trp Thr Asp Ser Asp Ala Leu Ala Leu Ser Gly Val Met
385 390 395 400

Val Ser Met Glu Glu Pro Gly Trp Tyr Ser Phe Gly Phe Glu Ile Lys
405 410 415

Asp Lys Lys Cys Asp Val Pro Cys Ile Gly Ile Glu Met Val His Asp
420 425 430

Gly Gly Lys Glu Thr Trp His Ser Ala Ala Thr Ala Ile Tyr Cys Leu
435 440 445

Met Gly Ser Gly Gln Leu Leu Trp Asp Thr Val Thr Gly Val Asn Met
450 455 460

Ala Leu
465

<210> 34

<211> 248

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 34

Met Ser Leu Phe Gly Asp Thr Ile Ala Tyr Leu Leu Ser Leu Thr Glu
1 5 10 15

Printed: 03-12-2004

SPEC AT

0012089

Asp Gly Glu Gly Lys Ala Glu Leu Ala Glu Lys Leu His Cys Trp Phe
20 25 30

Gly Gly Lys Glu Phe Asp Leu Asp Ser Ala Leu Glu Trp Ile Lys Asn
35 40 45

Lys Arg Cys Leu Thr Asp Ile Gln Lys Ala Leu Ile Gly Ala Ser Ile
50 55 60

Cys Phe Leu Lys Pro Lys Asp Gln Glu Arg Lys Arg Arg Phe Ile Thr
65 70 75 80

Glu Pro Leu Ser Gly Met Gly Thr Thr Ala Thr Lys Lys Lys Gly Leu
85 90 95

Ile Leu Ala Glu Arg Lys Met Arg Arg Cys Val Ser Phe His Glu Ala
100 105 110

Phe Glu Ile Ala Glu Gly His Glu Ser Ser Ala Leu Leu Tyr Cys Leu
115 120 125

Met Val Met Tyr Leu Asn Pro Gly Asn Tyr Ser Met Gln Val Lys Leu
130 135 140

Gly Thr Leu Cys Ala Leu Cys Glu Lys Gln Ala Ser His Ser His Arg
145 150 155 160

Ala His Ser Arg Ala Ala Arg Ser Ser Val Pro Gly Val Arg Arg Glu
165 170 175

Met Gln Met Val Ser Ala Met Asn Thr Ala Lys Thr Met Asn Gly Met
180 185 190

Gly Lys Gly Glu Asp Val Gln Lys Leu Ala Glu Glu Leu Gln Ser Asn
195 200 205

Ile Gly Val Leu Arg Ser Leu Gly Ala Ser Gln Lys Asn Gly Glu Gly
210 215 220

Ile Ala Lys Asp Val Met Glu Val Leu Lys Gln Ser Ser Met Gly Asn
225 230 235 240

Ser Ala Leu Val Lys Lys Tyr Leu
245

<210> 35

Printed 04-12-2000

SP20

00120898

<211> 109

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 35

Met Leu Glu Pro Phe Gln Ile Leu Ser Ile Cys Ser Phe Ile Leu Ser
1 5 10 15

Ala Leu His Phe Val Ala Trp Thr Ile Gly His Leu Asn Gln Ile Lys
20 25 30

Arg Gly Val Asn Met Lys Ile Arg Ile Lys Ser Pro Asn Lys Glu Thr
35 40 45

Ile Asn Arg Glu Val Ser Ile Leu Arg His Ser Tyr Gln Lys Glu Ile
50 55 60

Gln Ala Lys Glu Thr Met Lys Glu Val Leu Ser Asp Asn Met Glu Val
65 70 75 80

Leu Gly Asp His Ile Val Ile Glu Gly Leu Ser Ala Glu Glu Ile Ile
85 90 95

Lys Met Gly Glu Thr Val Leu Glu Ile Glu Glu Leu His
100 105

<210> 36

<211> 281

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 36

Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Val Gly Pro Gly Ala
1 5 10 15

Thr Asn Ala Thr Ile Asn Phe Glu Thr Gly Ile Leu Glu Cys Tyr Glu
20 25 30

Arg Leu Ser Trp Gln Arg Ala Leu Asp Tyr Pro Gly Gln Asp Arg Leu
35 40 45

Asn Arg Leu Lys Arg Lys Leu Glu Ser Arg Ile Lys Thr His Asn Lys
50 55 60

Ser Glu Pro Glu Ser Lys Arg Met Ser Leu Glu Glu Arg Lys Ala Ile
65 70 75 80

Printed: 08-12-2001

SPECIAL

0012089

Gly Val Lys Met Met Lys Val Leu Leu Phe Met Asn Pro Ser Ala Gly
85 90 95

Ile Glu Gly Phe Glu Pro Tyr Tyr Met Lys Ser Ser Ser Asn Ser Asn
100 105 110

Cys Pro Lys Tyr Asn Trp Thr Asp Tyr Pro Ser Thr Pro Gly Arg Cys
115 120 125

Leu Asp Asp Ile Glu Glu Glu Pro Glu Asp Val Asp Gly Pro Thr Glu
130 135 140

Ile Val Leu Arg Asp Met Asn Asn Lys Asp Ala Arg Gln Lys Ile Lys
145 150 155 160

Glu Glu Val Asn Thr Gln Lys Glu Gly Lys Phe Arg Leu Thr Ile Lys
165 170 175

Arg Asp Ile Arg Asn Val Leu Ser Leu Arg Val Leu Val Asn Gly Thr
180 185 190

Phe Leu Lys His Pro Asn Gly Tyr Lys Ser Leu Ser Thr Leu His Arg
195 200 205

Leu Asn Ala Tyr Asp Gln Ser Gly Arg Leu Val Ala Lys Leu Val Ala
210 215 220

Thr Asp Asp Leu Thr Val Glu Asp Glu Glu Asp Gly His Arg Ile Leu
225 230 235 240

Asn Ser Leu Phe Glu Arg Leu Asn Glu Gly His Ser Lys Pro Ile Arg
245 250 255

Ala Ala Glu Thr Ala Val Gly Val Leu Ser Gln Phe Gly Gln Glu His
260 265 270

Arg Leu Ser Pro Glu Glu Gly Asp Asn
275 280

<210> 37
<211> 122
<212> PRT
<213> Influenza B/Vienna/1/99/as

<400> 37
Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Trp Arg Met Lys Lys
1 5 10 15

Printed 03-12-2000

SPEC

00120898

Met Ala Ile Gly Ser Ser Thr His Ser Ser Ser Val Leu Met Lys Asp
20 25 30

Ile Gln Ser Gln Phe Glu Gln Leu Lys Leu Arg Trp Glu Ser Tyr Pro
35 40 45

Asn Leu Val Lys Ser Thr Asp Tyr His Gln Lys Arg Glu Thr Ile Lys
50 55 60

Leu Val Thr Glu Glu Leu Tyr Leu Leu Ser Lys Arg Ile Asp Asp Asn
65 70 75 80

Ile Leu Phe His Lys Thr Val Ile Ala Asn Ser Ser Ile Ile Ala Asp
85 90 95

Met Val Val Ser Leu Ser Leu Leu Glu Thr Leu Tyr Glu Met Lys Asp
100 105 110

Val Val Glu Val Tyr Ser Arg Gln Cys Leu
115 120